

Amino acid sequence of human mature IFN $\alpha$ -2b

1	10	20	30	40	50
IFN $\alpha$ -2b	<u>CDLPQTHSLGSRRTLMLLAQMRRI SLFSCLKDRHDFGFPQEEFGNQFQKA</u>				
51	60	70	80	90	100
IFN $\alpha$ -2b	<u>ETIPVLHEMIQQIFNLSTKDSAAWDETLLDKFYTELYQQQLNDLEACVI</u>				
101	110	120	130	140	150
IFN $\alpha$ -2b	<u>QGVGVTTETPLMKEDSILAVRKYFQRI TLYLKEKKYSPCAWEVVRAEIMRS</u>				
151	160				
IFN $\alpha$ -2b	<u>FSLSTNLQESLRSKE</u>				

FIG.1A

Three dimensional structure of INF $\alpha$ -2b

showing candidate LEADs

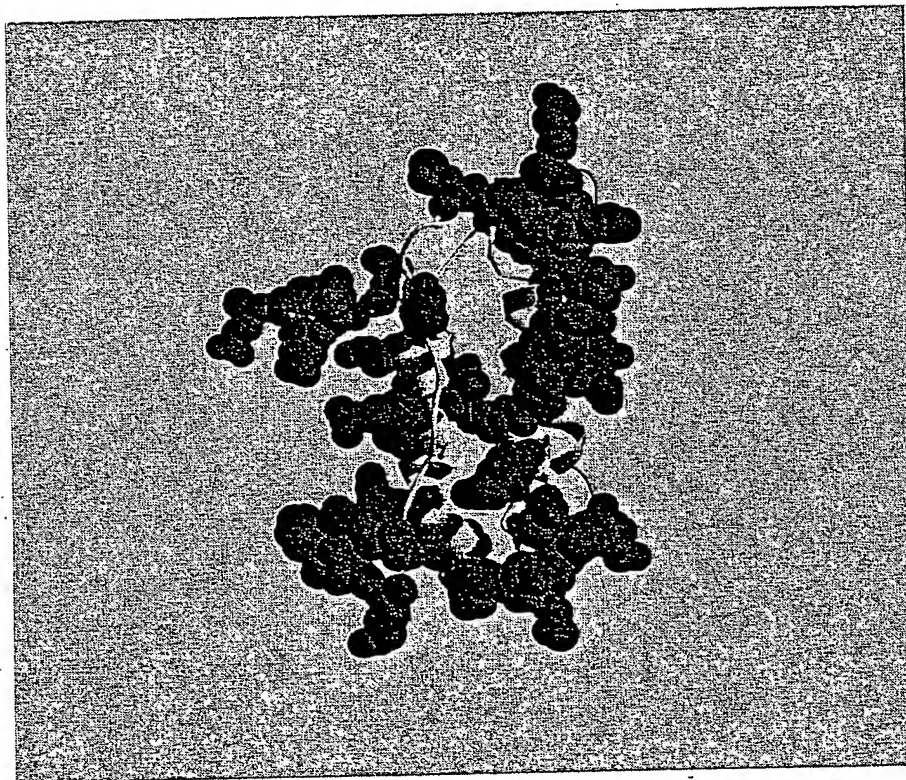


FIG.1B

The "Percent Accepted Mutation" (PAM250) matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	-2	-2	0	0	-2	0	0	1	-1	-1	-2	-1	-1	-3	1	1	1	-6	-3	0
R	-2	6	0	-1	-4	1	-1	-3	2	-2	-3	3	0	-4	0	0	-1	2	-4	-2
N	0	0	2	2	-4	1	1	0	2	-2	-3	1	-2	-3	0	1	0	-4	-2	-2
D	0	-1	2	4	-5	2	3	1	1	-2	-4	0	-3	-6	-1	0	0	-7	-4	-2
C	-2	-4	-4	-5	12	-5	-5	-3	-3	-2	-6	-5	-5	-4	-3	0	-2	-8	0	-2
Q	0	1	1	2	-5	4	2	-1	3	-2	-2	1	-1	-5	0	-1	-1	-5	-4	-2
E	0	-1	1	3	-5	2	4	0	1	-2	-3	0	-2	-5	-1	0	0	-7	-4	-2
G	1	-3	0	1	-3	-1	0	5	-2	-3	-4	-2	-3	-5	0	1	0	-7	-5	-1
H	-1	2	2	1	-3	3	1	-2	6	-2	-2	0	-2	-2	0	-1	-1	-3	0	-2
I	-1	-2	-2	-2	-2	-2	-2	-3	-2	5	2	-2	2	1	-2	-1	0	-5	-1	4
L	-2	-3	-3	-4	-6	-2	-3	-4	-2	2	6	-3	4	2	-3	-3	-2	-2	-1	2
K	-1	3	1	0	-5	1	0	-2	0	-2	-3	5	0	-5	-1	0	0	-3	-4	-2
M	-1	0	-2	-3	-5	-1	-2	-3	-2	2	4	0	6	0	-2	-2	-1	-4	-2	2
F	-3	-4	-3	-6	-4	-5	-5	-5	-2	1	2	-5	0	9	-5	-3	-3	0	7	-1
P	1	0	0	-1	-3	0	-1	0	0	-2	-3	-1	-2	-5	6	1	0	-6	-5	-1
S	1	0	1	0	0	-1	0	1	-1	-1	-3	0	-2	-3	1	2	1	-2	-3	-1
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-3	0	1	3	-5	-3	0
W	-6	2	-4	-7	-8	-5	-7	-7	-3	-5	-2	-3	-4	0	-6	-2	-5	17	0	-6
Y	-3	-4	-2	-4	0	-4	-4	-5	0	-1	-1	-4	-2	7	-5	-3	-3	0	10	-2
V	0	-2	-2	-2	-2	-2	-2	-1	-2	4	2	-2	2	-1	-1	-1	0	-6	-2	4

FIG.2

Scores from PAM250, given to residue substitutions to  
protect human INF  $\alpha$ -2b against proteolysis

	R	D	E	F	K	M	F	P	W	Y
A	-2	0	0	-2	-1	-1	-3	<b>1</b>	-6	-3
N	0	<b>2</b>	<b>1</b>	-3	<b>1</b>	-2	-3	0	-4	-2
C	-4	-5	-5	-6	-5	-5	-4	-3	-8	0
Q	<b>1</b>	<b>2</b>	<b>2</b>	-2	<b>1</b>	-1	-5	0	-5	-4
G	-3	1	0	-4	-2	-3	-5	0	-7	-5
H	<b>2</b>	1	<b>1</b>	-2	0	-2	-2	0	<b>-3</b>	<b>0</b>
I	-2	-2	-2	<b>2</b>	-2	<b>2</b>	<b>1</b>	-2	-5	<b>-1</b>
S	0	0	0	-3	0	-2	-3	<b>1</b>	<b>-2</b>	-3
T	-1	0	0	-2	0	-1	-3	0	-5	-3
V	-2	-2	-2	<b>2</b>	-2	<b>2</b>	<b>-1</b>	-1	-6	-2

FIG.3

Protection against proteolysis for interferon  $\alpha$ -2b variants

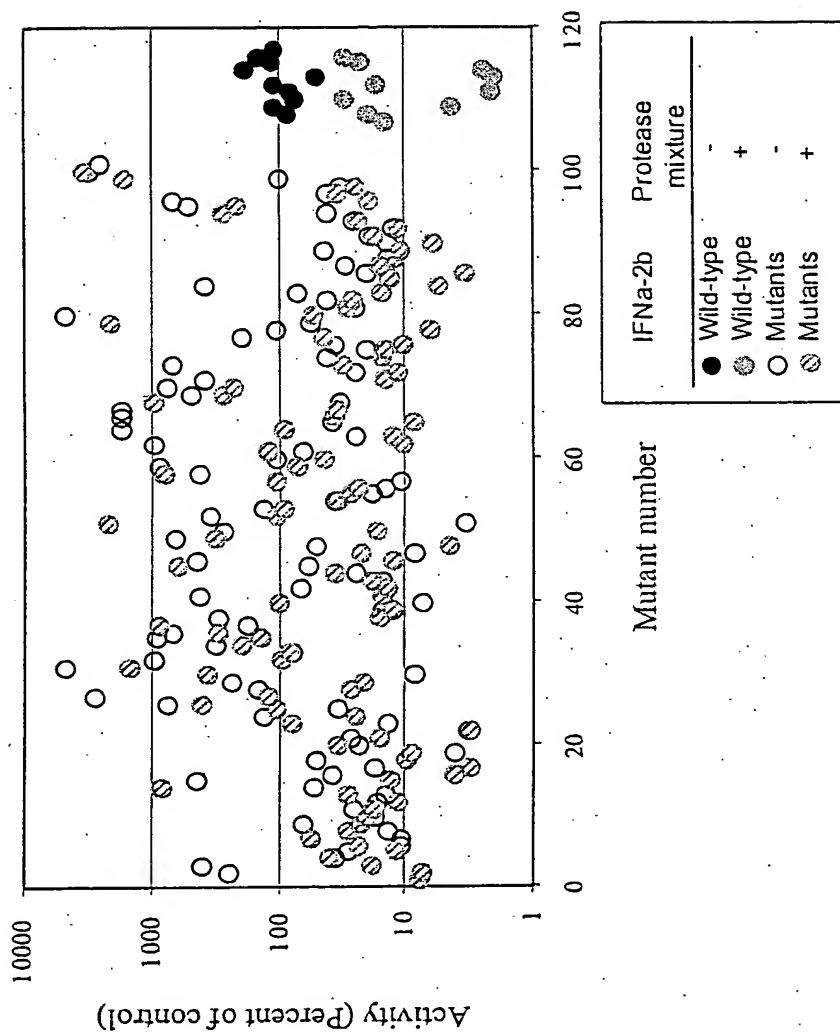


FIG.4A

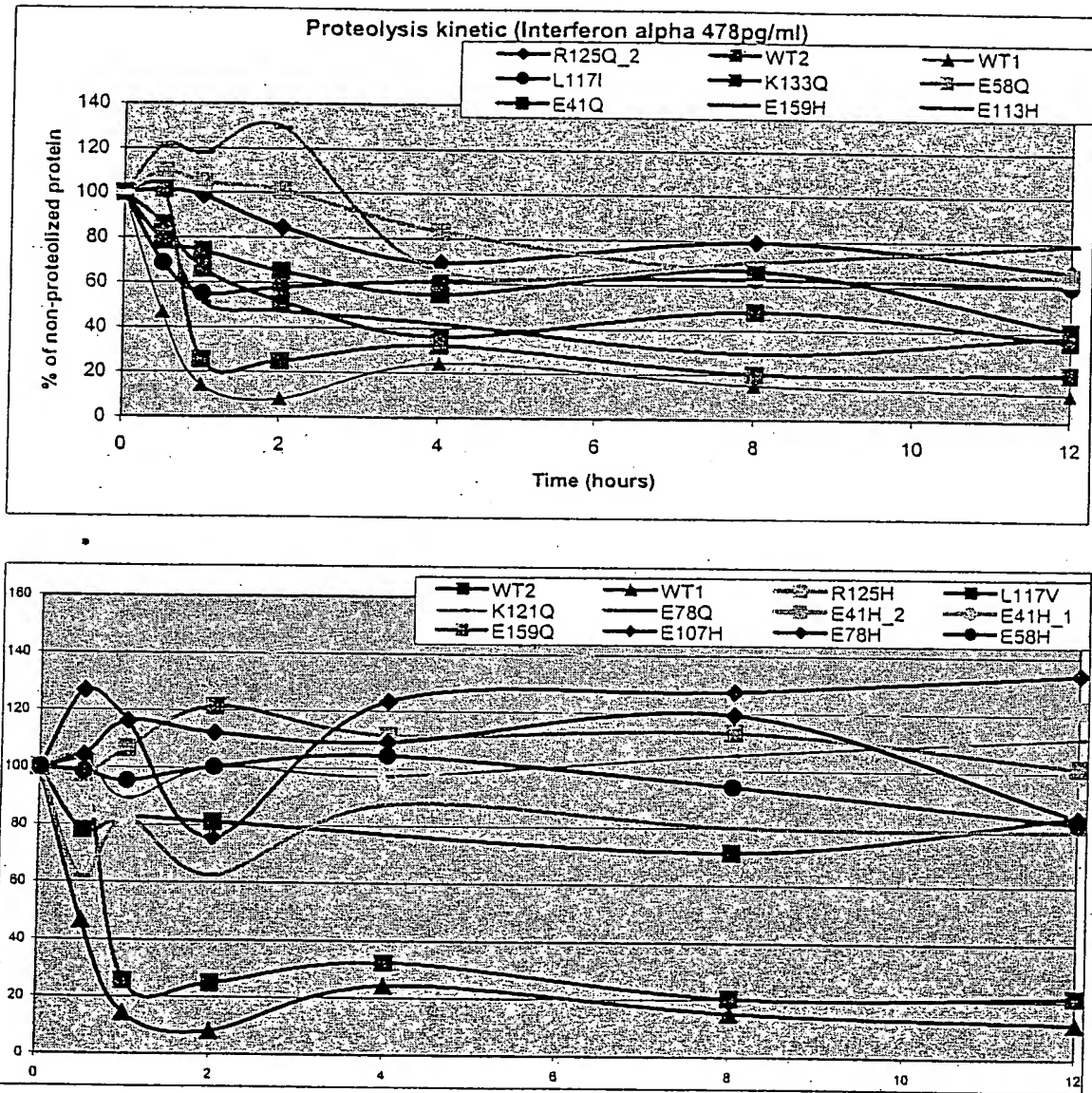


FIG.4B

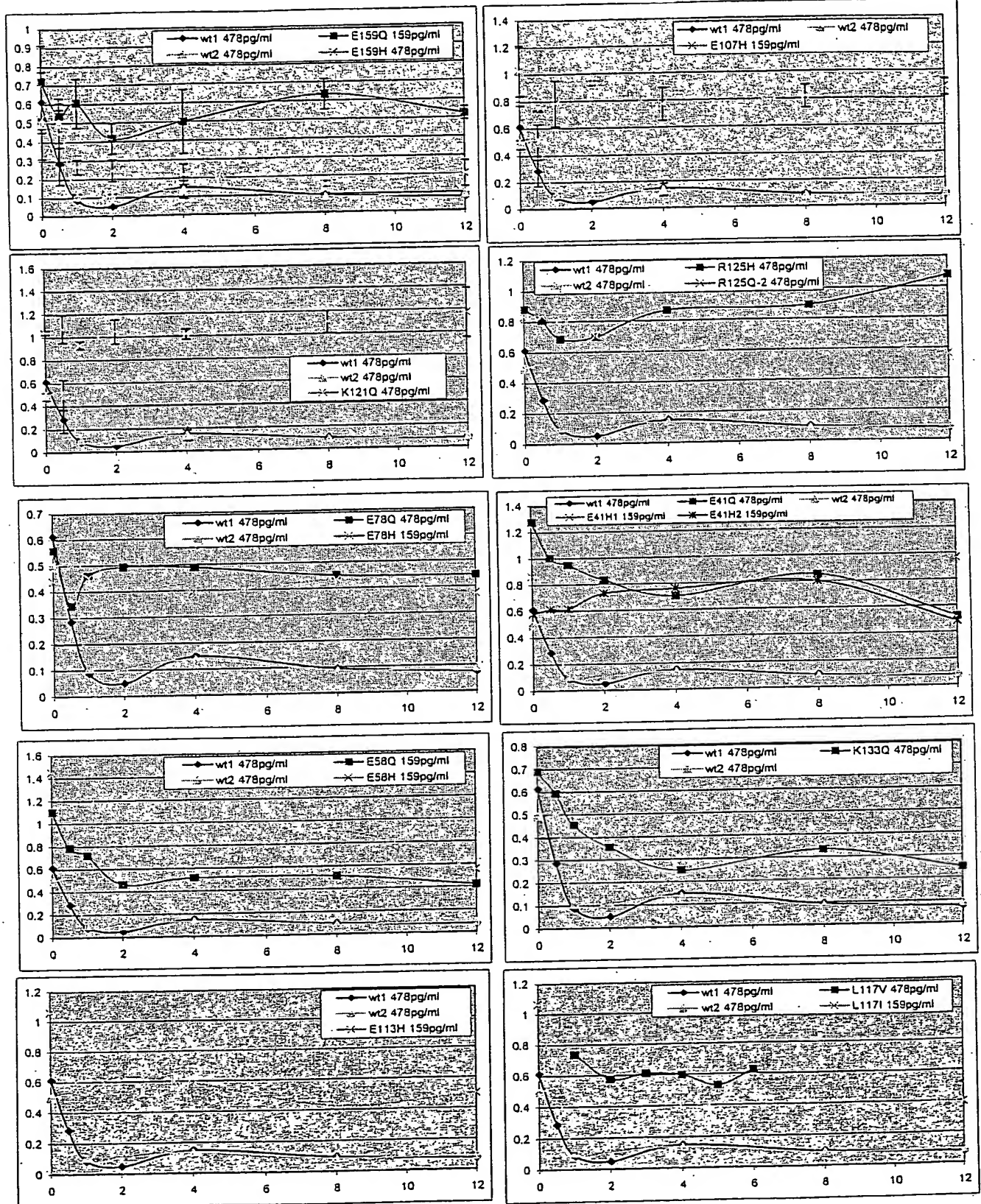


FIG.4C

Treatment with chymotrypsin

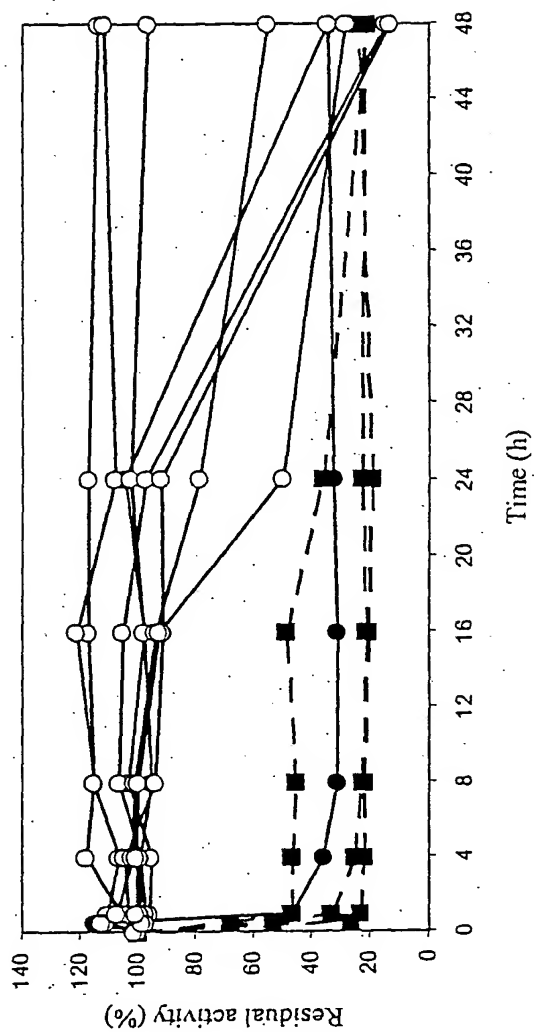


FIG.5



### Treatment with chymotrypsin

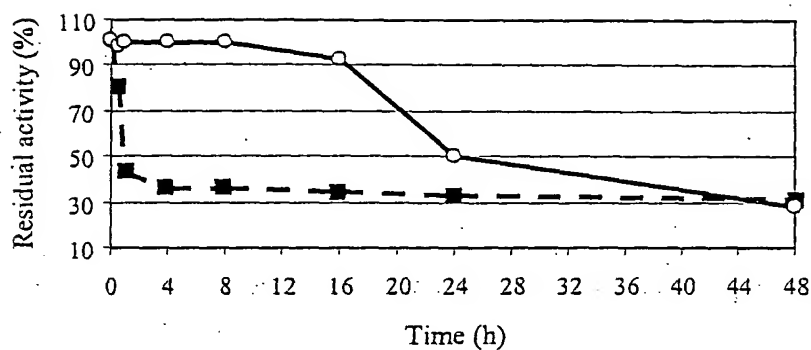


FIG.6A

### Treatment with protease mixture

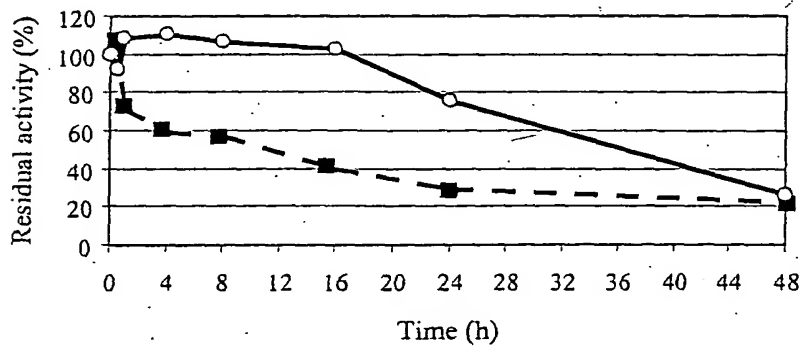


FIG.6B

Treatment with blood lysate

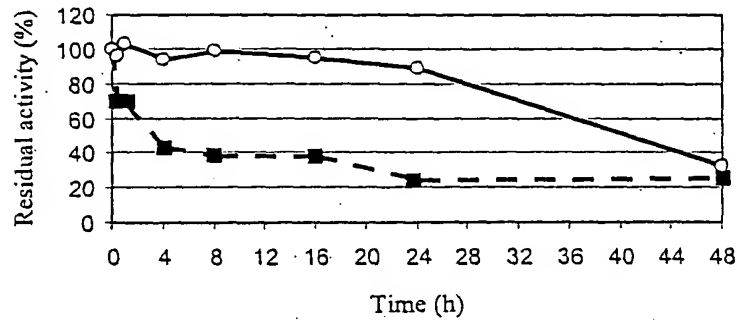


FIG.6C

Treatment with serum

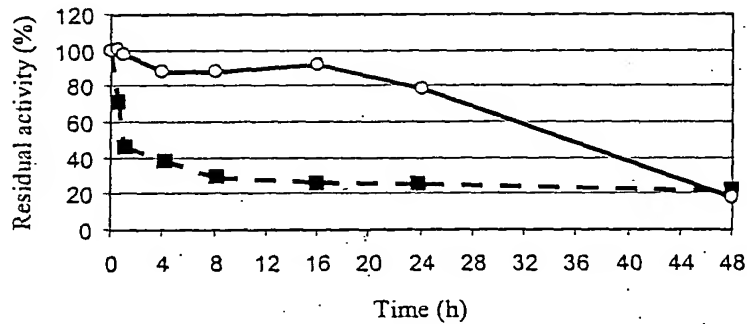


FIG.6D

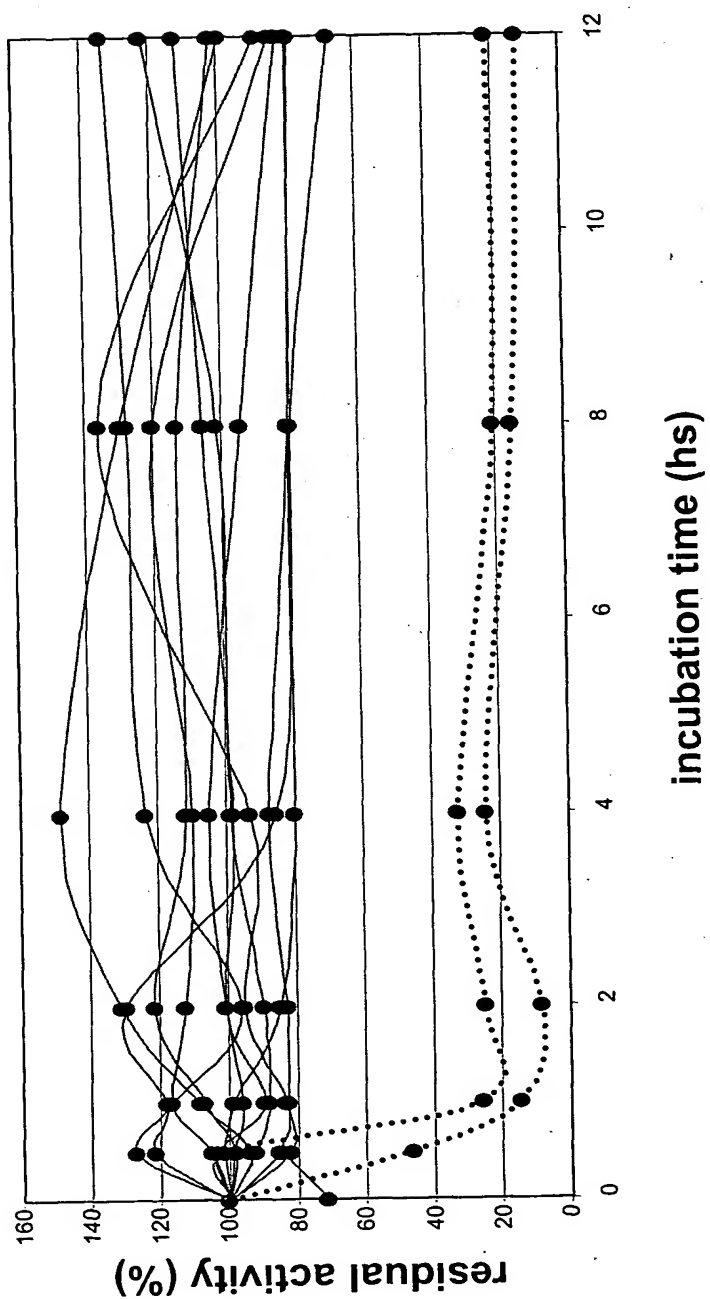


FIG. 6(E)

Title:

RATIONAL EVOLUTION OF CYTOKINE  
HIGHER STABILITY, THE CYTOKINES  
ENCODING NUCLEIC ACID MOLECULE

Docket No.: 37851-922

Applicant: Gantier et al.

Filed: September 8 2003

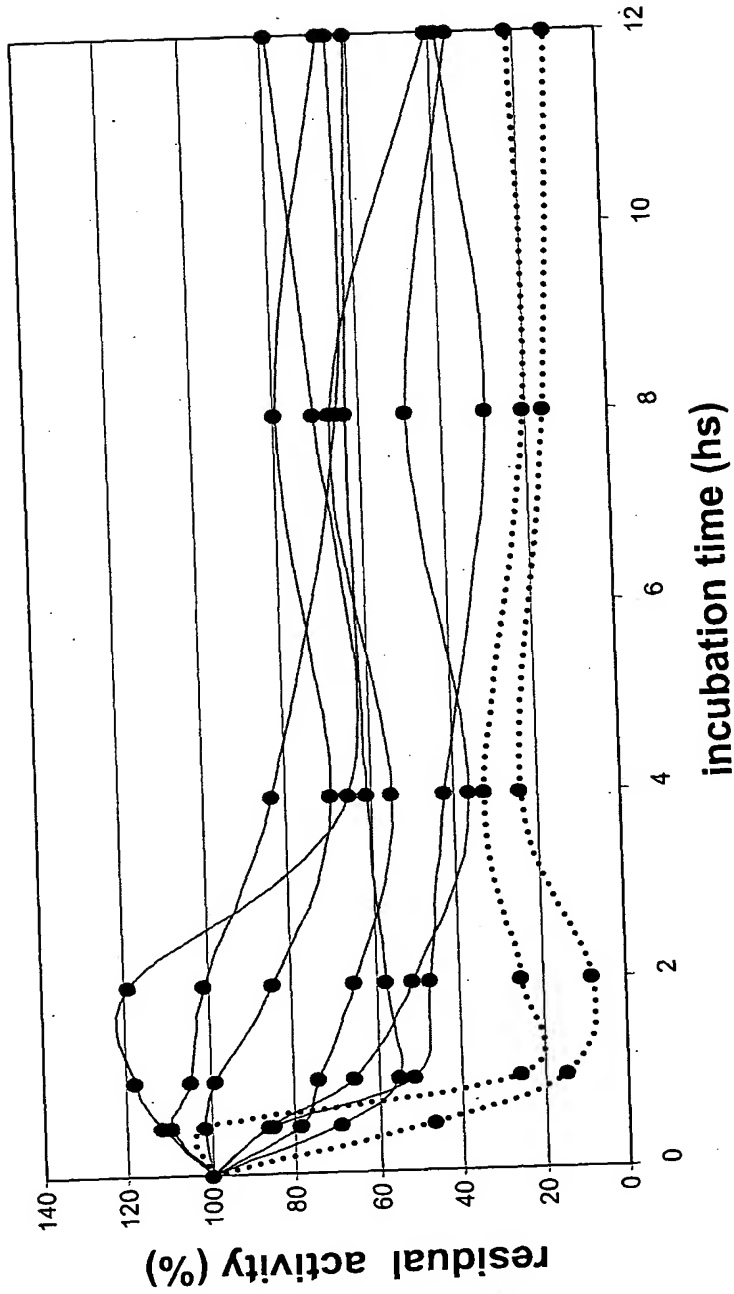


FIG. 6(F)

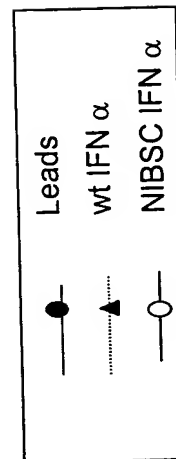
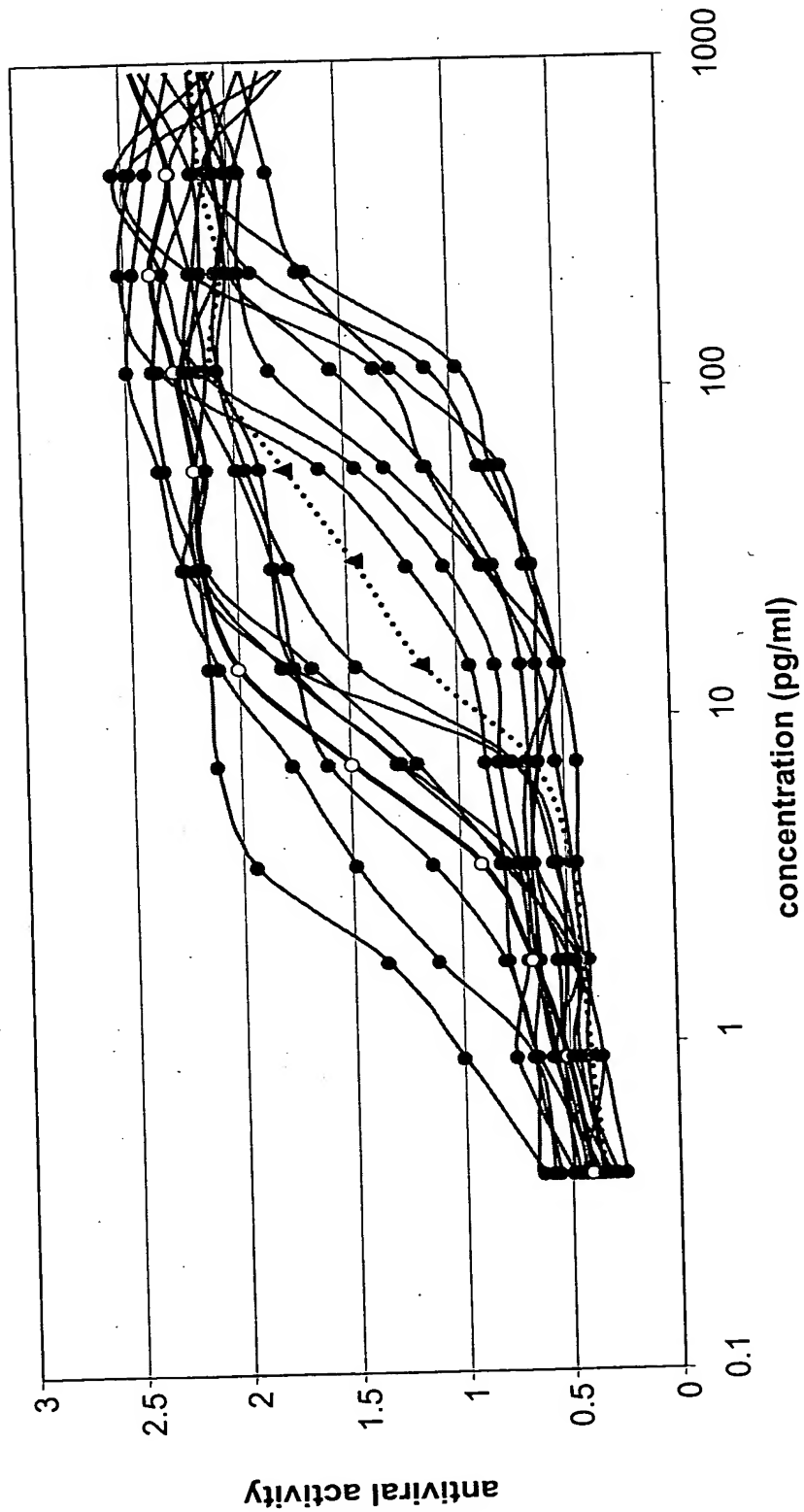
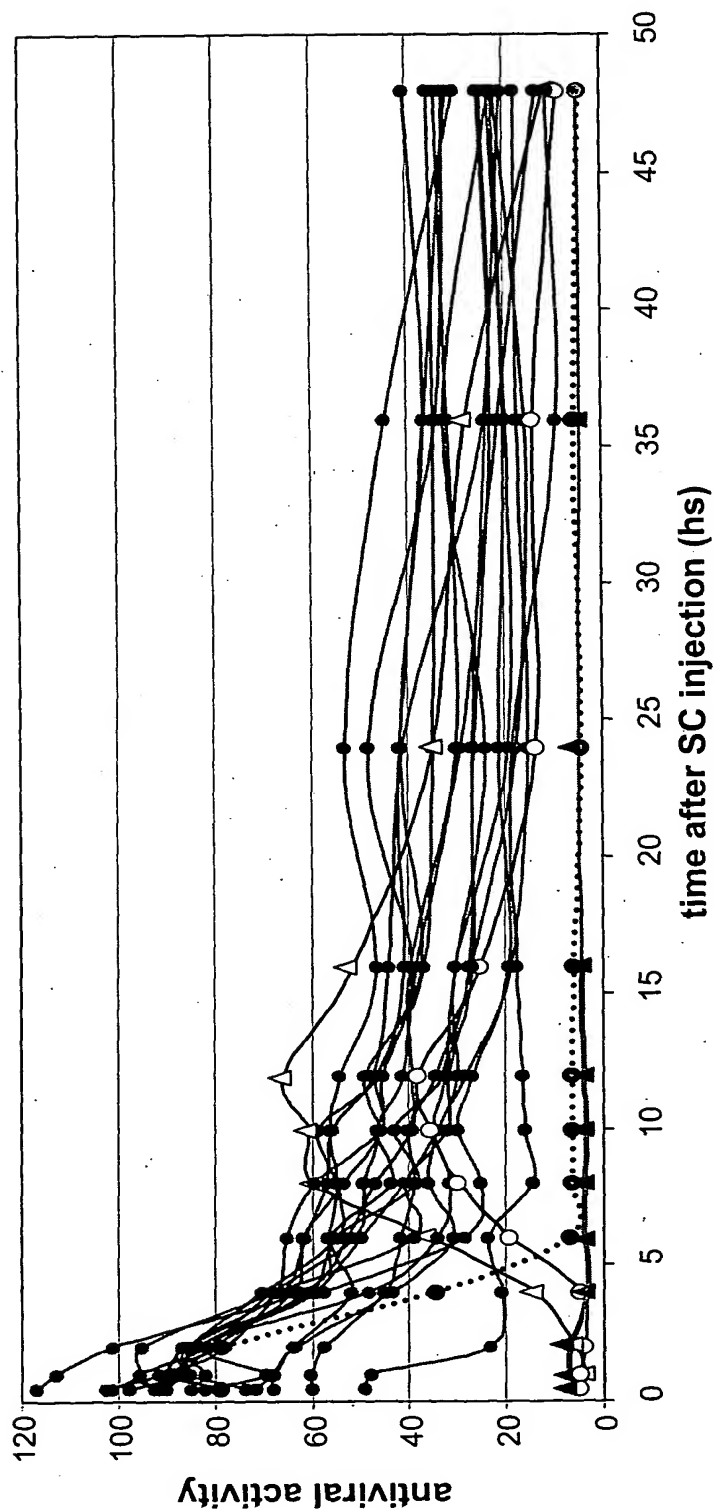


FIG. 6(G)

## Potency (antiproliferation) – IFN $\alpha$ leads

	Potency (10 <sup>8</sup> U/mg)
WT	1,70
Lead 13	1,60
Lead 9	1,90
Lead 8	2,05
Lead 2	3,70
Lead 16	1,60
Lead 4	0,50
Lead 5	0,65
Lead 15	3,20
Lead 10	0,50
Lead 12	1,50
Lead 11	nd
Lead 6	1,20
Lead 1	2,95
Lead 7	1,60
Lead 3	2,25
Lead 14	nd

Fig. 6(H)



Reference to Figures 6I, 6K-N

- Leads
- △ Pegasys 36  $\mu$ g / ml
- ⋯ wt IFN  $\alpha$
- Pegasys 18  $\mu$ g / ml
- ▲ vehicle

FIG. 6(I)

## FIGURE 6(J)

### IFN- $\alpha$ LEADS

IFN- $\alpha$ LEAD	SEQ ID N°	Mutation(s)
1	983	K121Q / P109A
2	987	E159H / Y89H
3	124	E159Q
4	90	E58H
5	89	E58Q
6	979	E41H / Y89H / N45D
7	103	L117I
8	986	R125H / M111V
9	96	E107H
10	101	E113H
11	87	E41Q
12	107	R125Q
13	985	L117V / A139G
14	980	E41Q / D94G
15	93	E78H
16	984	K133Q / K121Q / P109A / G102R



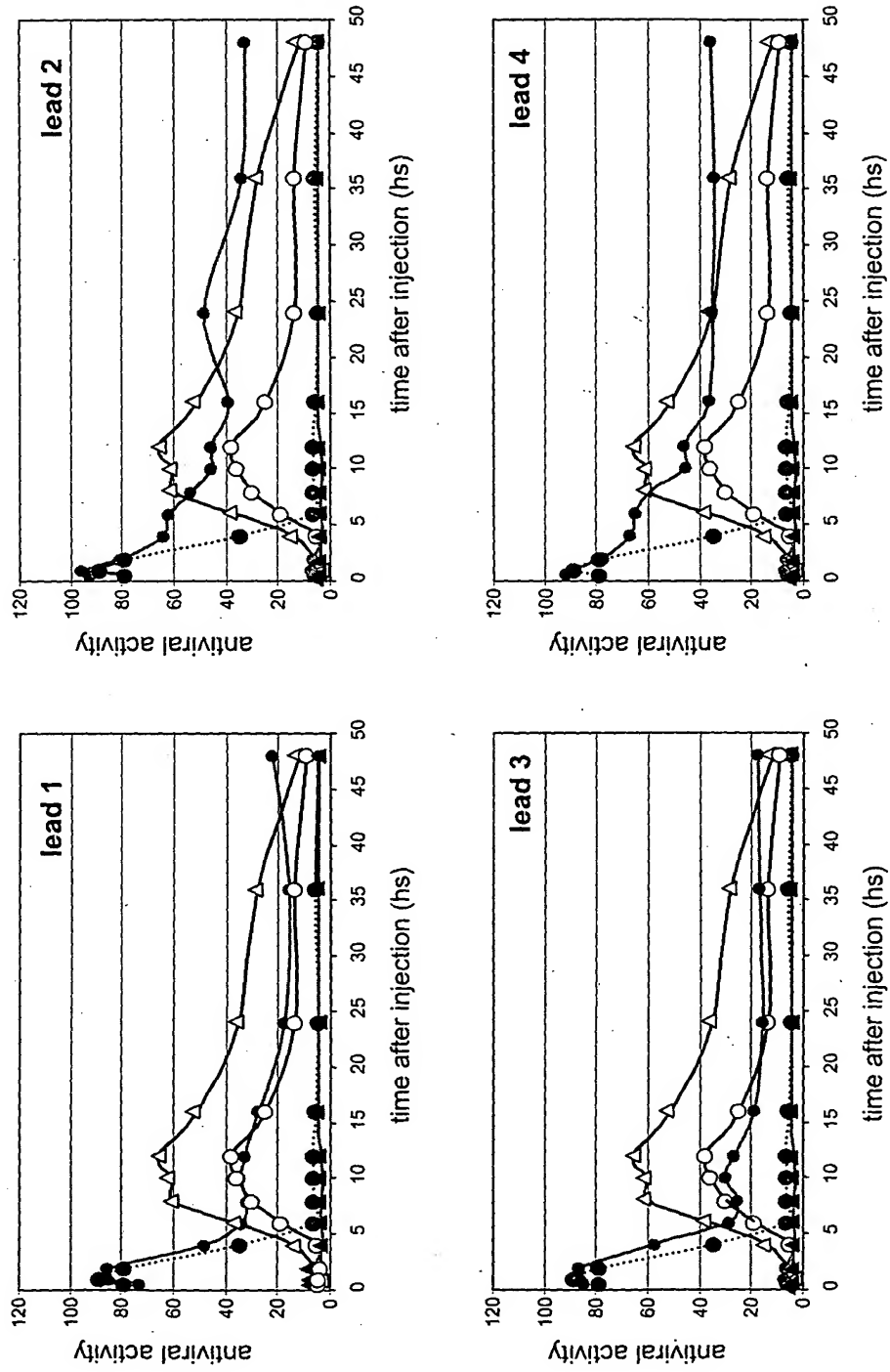
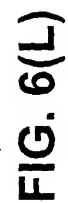


FIG. 6(K)



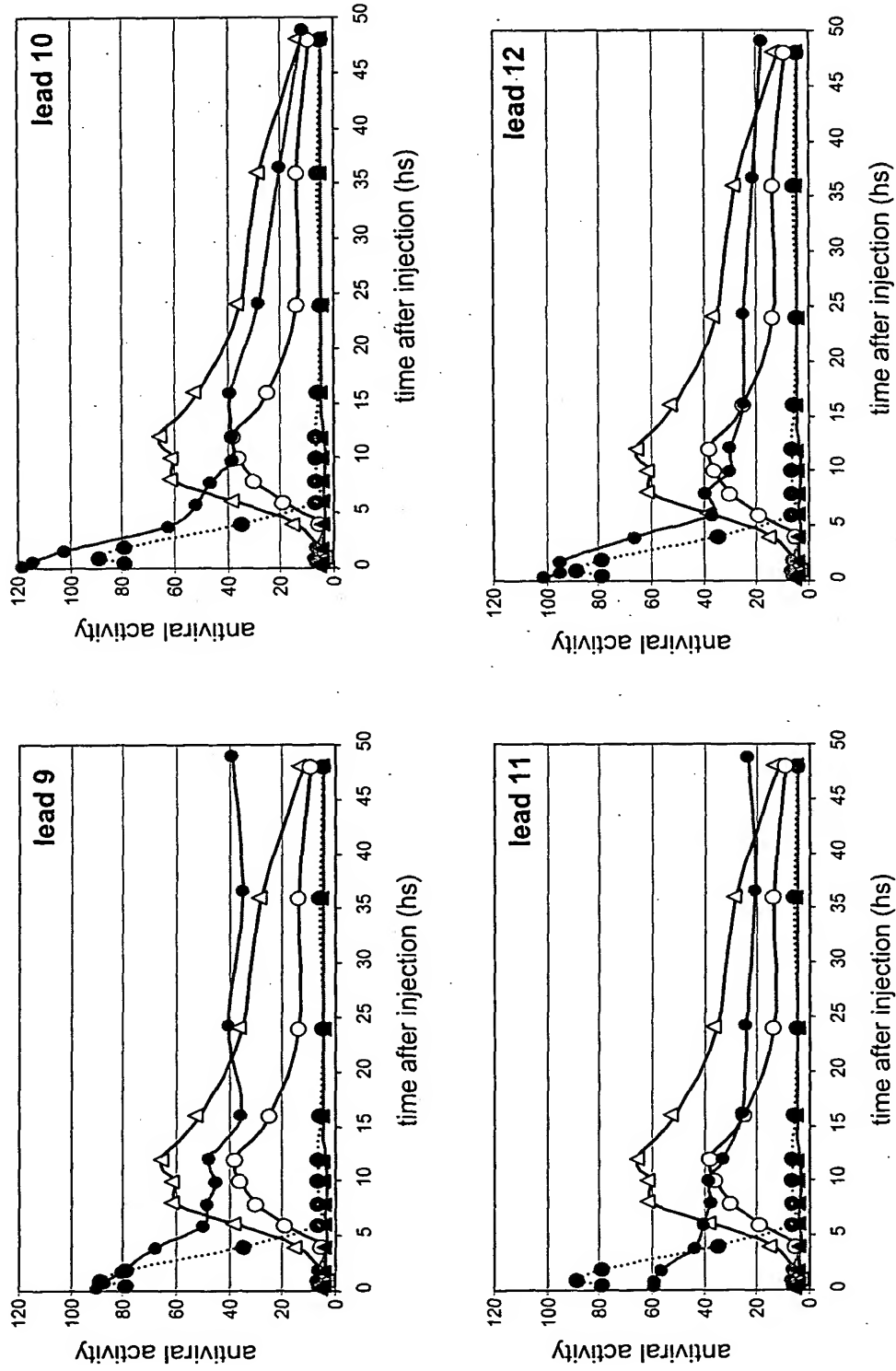


FIG. 6(M)

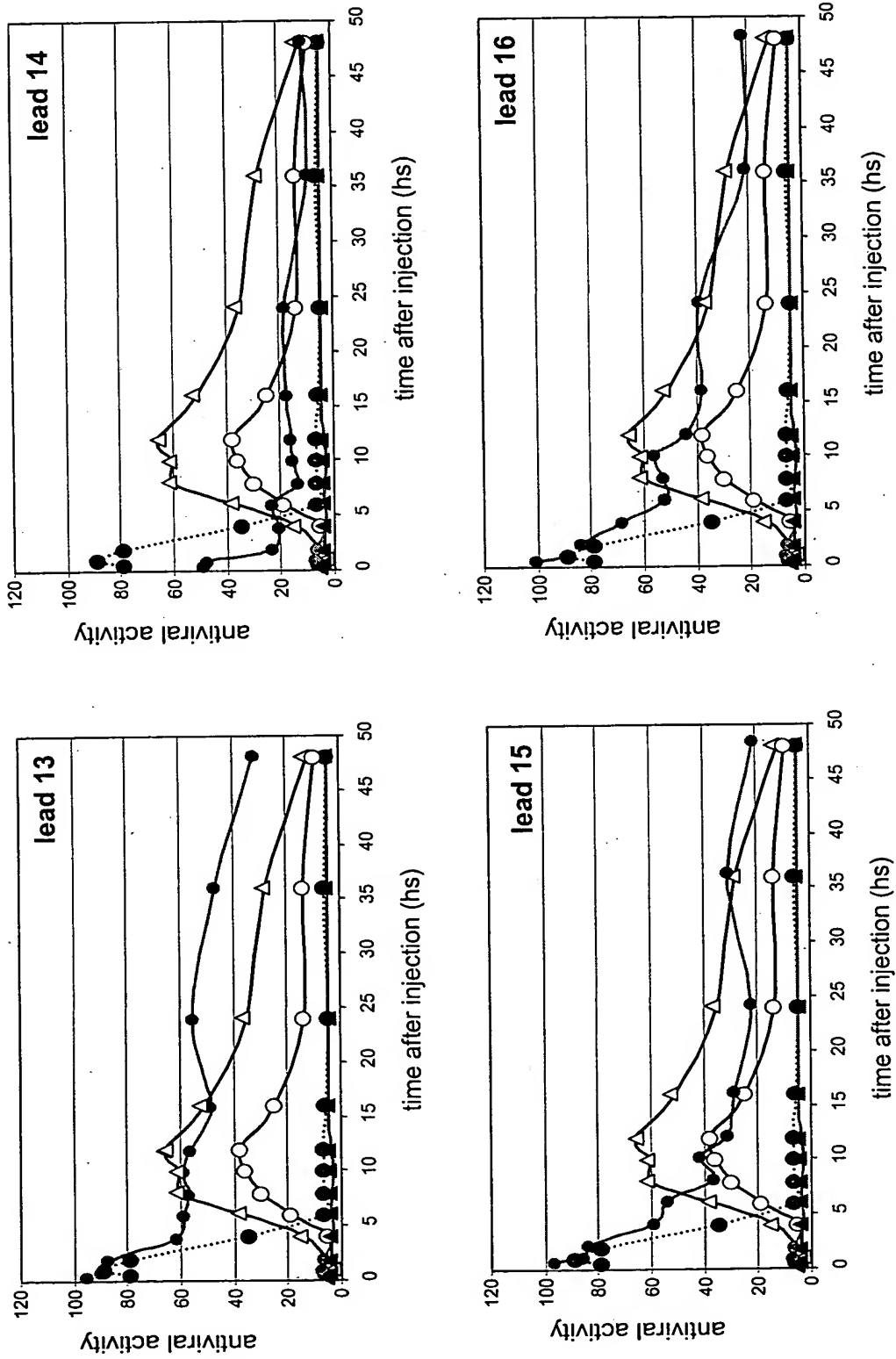
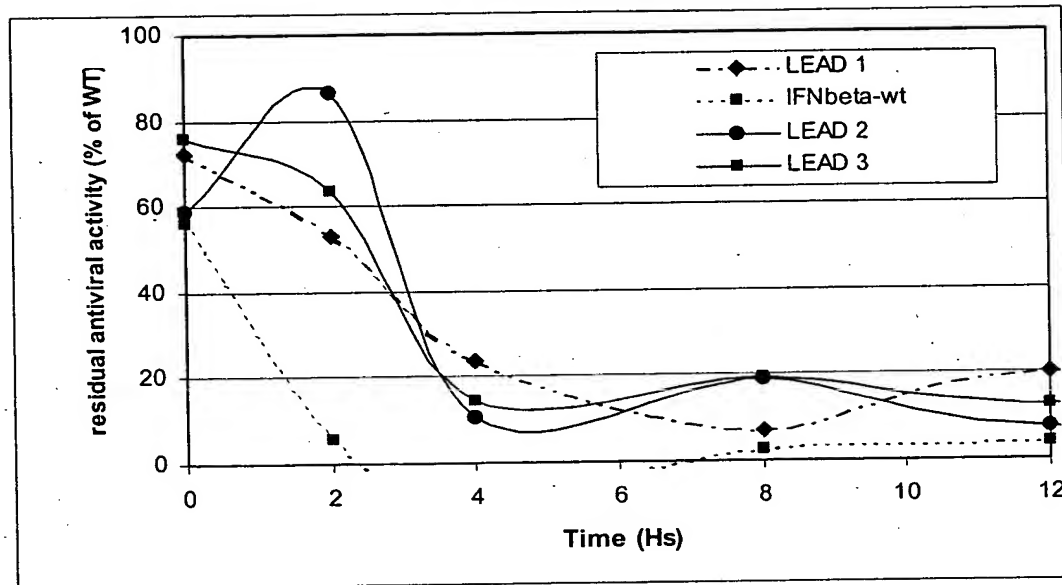


FIG. 6(N)



**FIG 6(O)**

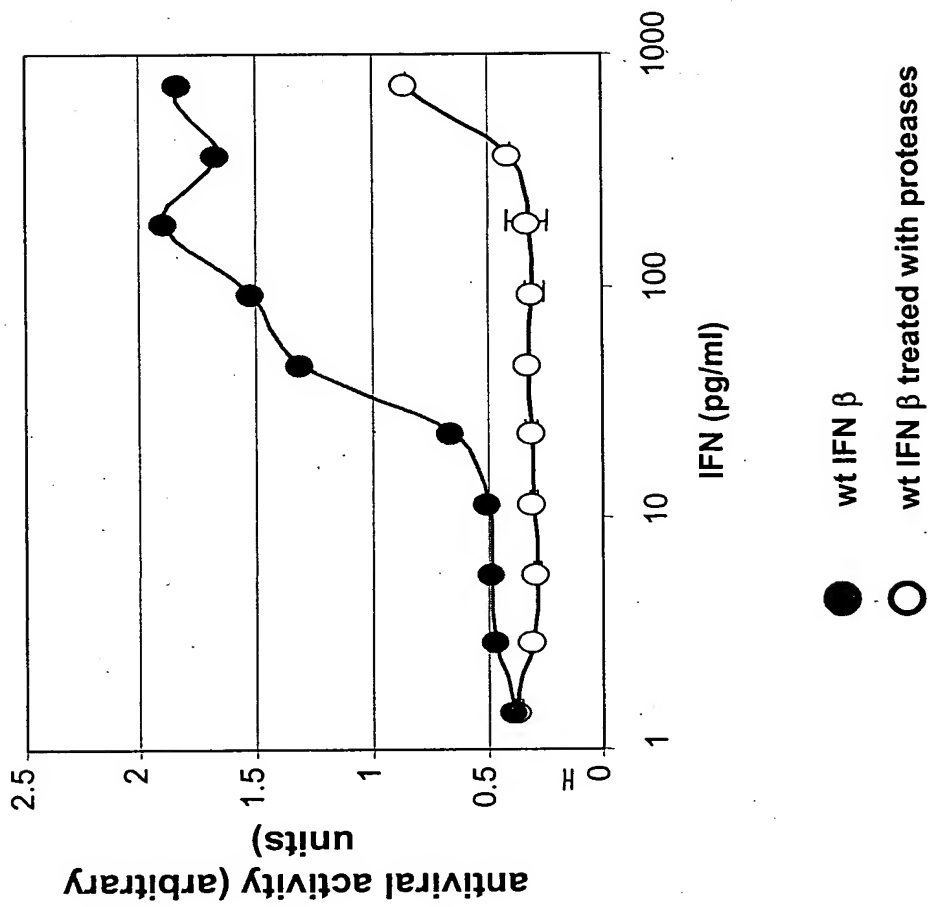


FIG. 6(P)

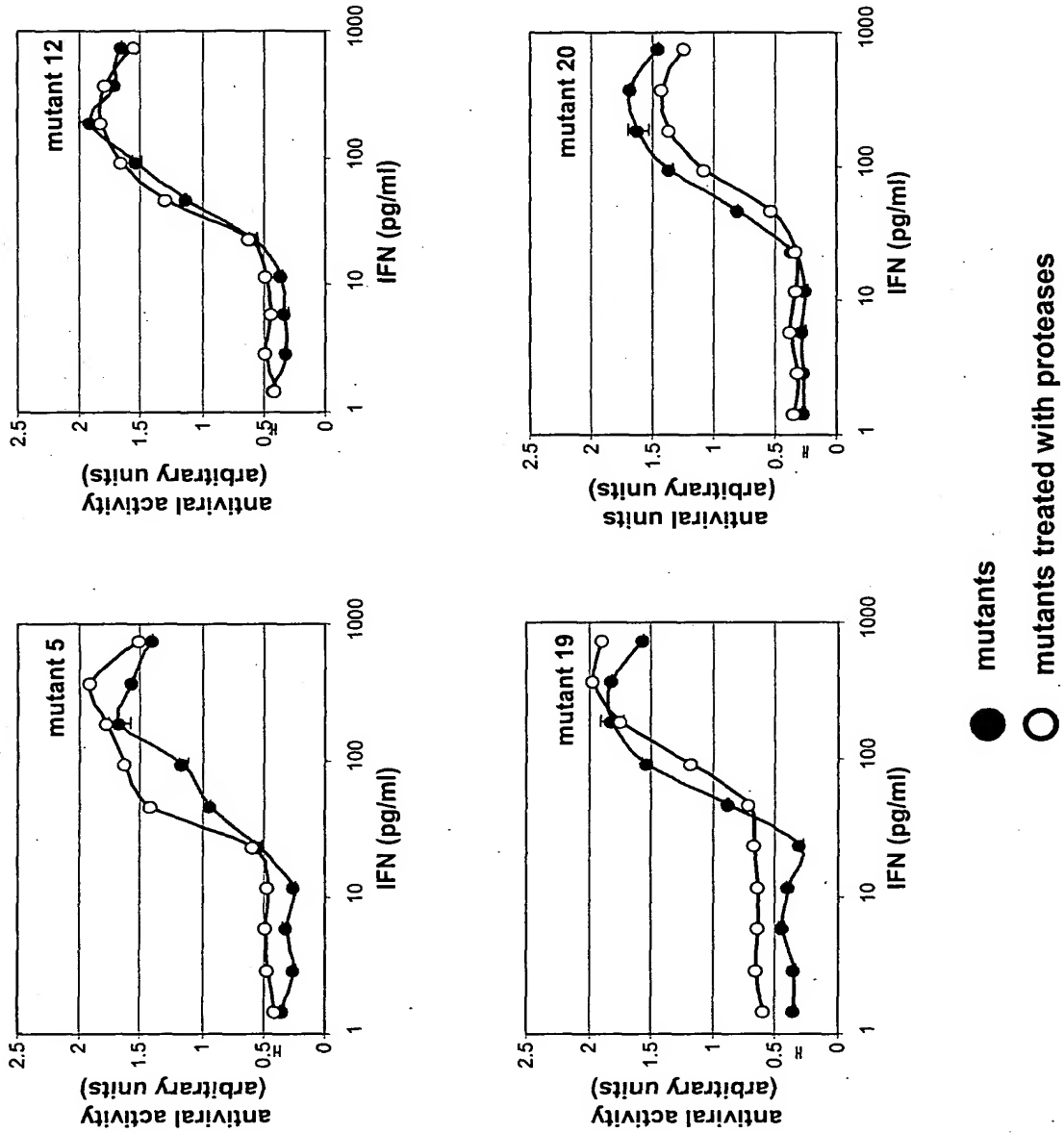


FIG. 6(Q)

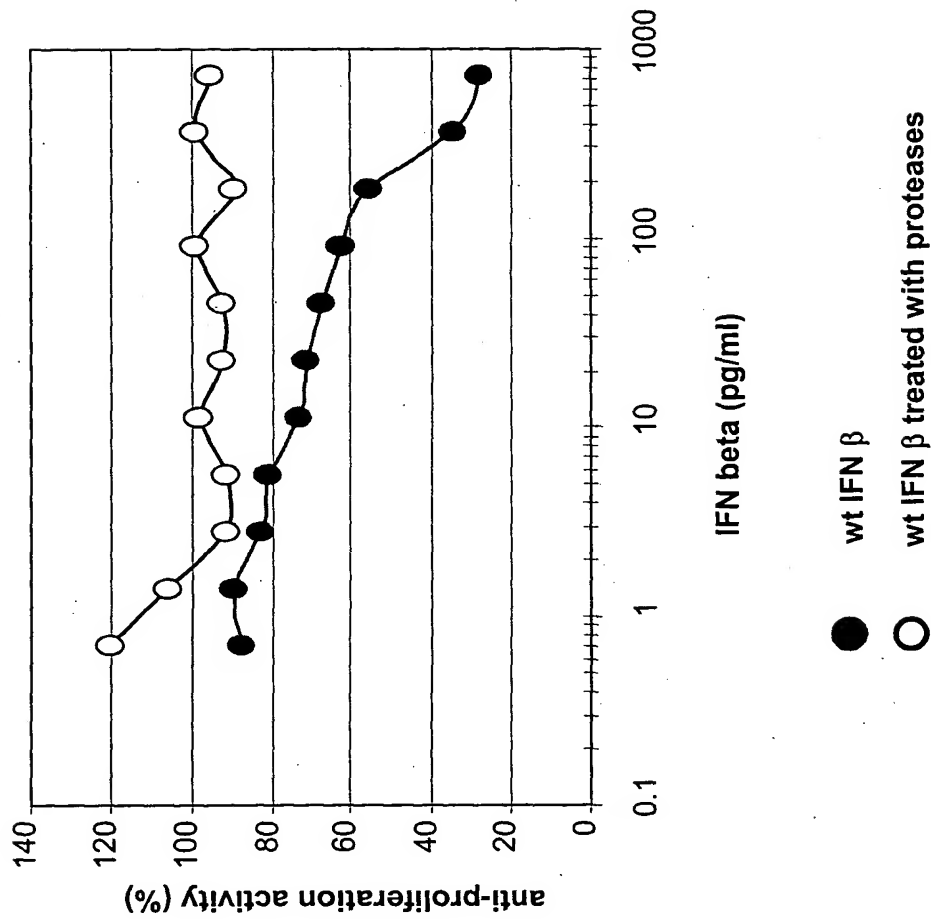


FIG. 6(R)



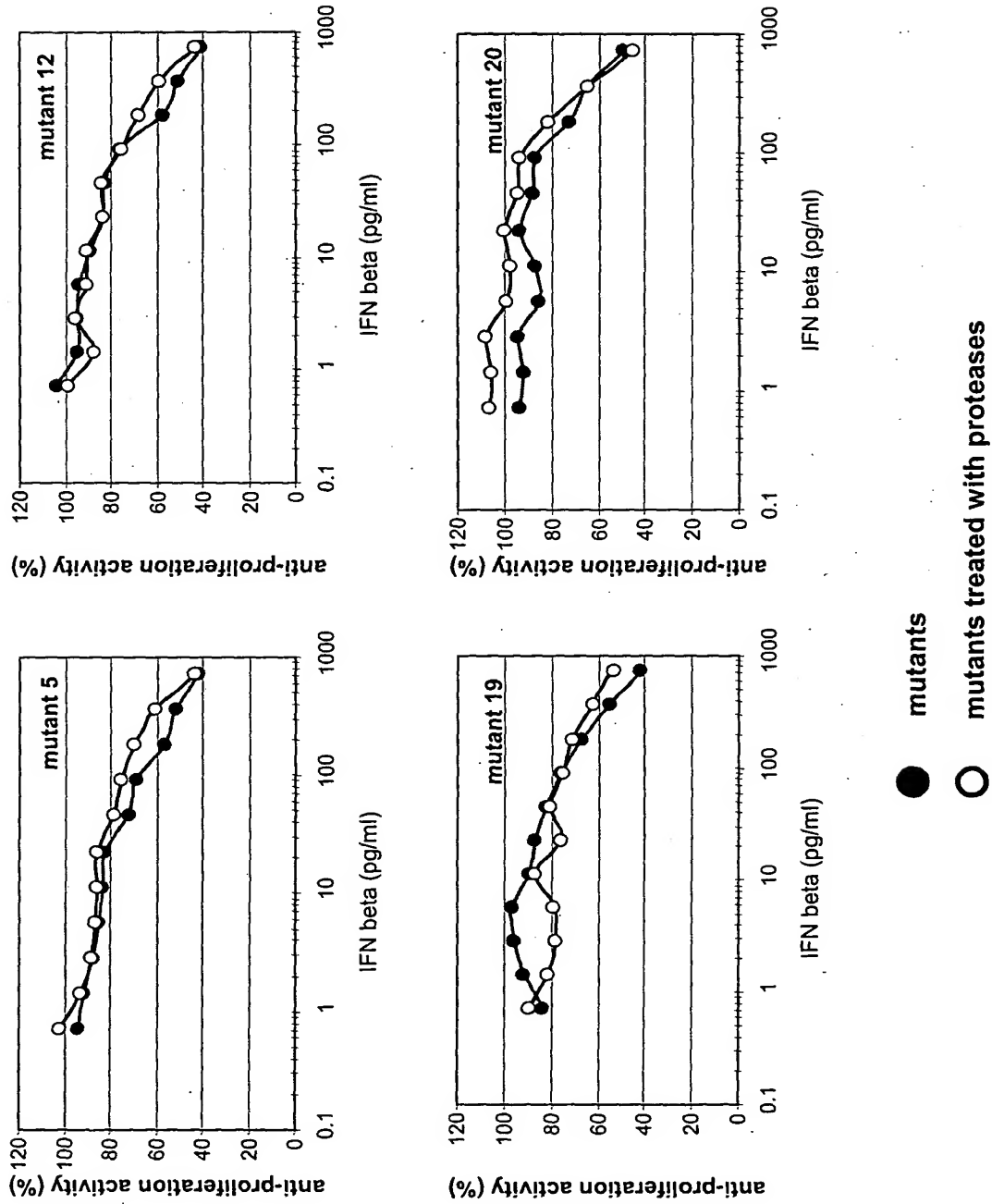


FIG. 6(S)

## Summary – IFN $\alpha$ leads

Fig. 6(T)

	Potency (AV) (10 <sup>8</sup> U/mg)	Potency (AP) (10 <sup>8</sup> U/mg)	AUC (arbitrary units)
WT	1,6	1,7	16,5
Pegasys			33,0
Pegasys			77,0
Lead 13	0,4	1,6	129,7
Lead 9	1,2	1,9	109,0
Lead 8	1,0	2,1	107,0
Lead 2	2,1	3,7	105,0
Lead 16	0,8	1,6	101,6
Lead 4	4,3	0,5	100,0
Lead 5	1,2	0,7	88,6
Lead 15	1,7	3,2	88,0
Lead 10	5,5	0,5	85,6
Lead 12	1,4	1,5	77,0
Lead 11	28,5	nd	69,0
Lead 6	1,7	1,2	64,2
Lead 1	1,9	3,0	58,5
Lead 7	1,7	1,6	56,5
Lead 3	1,7	2,3	54,6
Lead 14	0,9	nd	25,0

**Fig. 6(U) IFN $\alpha$  LEADS-- Area under the curve (AUC)**

	AUC (arbitrary units)	protein injected ( $\mu\text{g/ml}^*$ )	IFN units injected / ml ( $\times 10^6$ )
WT	16,5	2,5	2,0
Pegasys	33,0	18,0	
Pegasys	77,0	36,0	
Lead 13	129,7	10,3	2,0
Lead 9	109,0	3,5	2,0
Lead 8	107,0	4,2	2,0
Lead 2	105,0	2,0	2,0
Lead 16	101,6	5,4	2,0
Lead 4	100,0	1,0	2,0
Lead 5	88,6	3,6	2,0
Lead 15	88,0	2,4	2,0
Lead 10	85,6	1,0	2,0
Lead 12	77,0	3,0	2,0
Lead 11	69,0	0,2	2,0
Lead 6	64,2	3,4	2,0
Lead 1	58,5	2,1	2,0
Lead 7	56,5	2,4	2,0
Lead 3	54,6	2,5	2,0
Lead 14	25,0	2,0	2,0



Interferon  $\alpha$ -2b structure in "space filling" representation

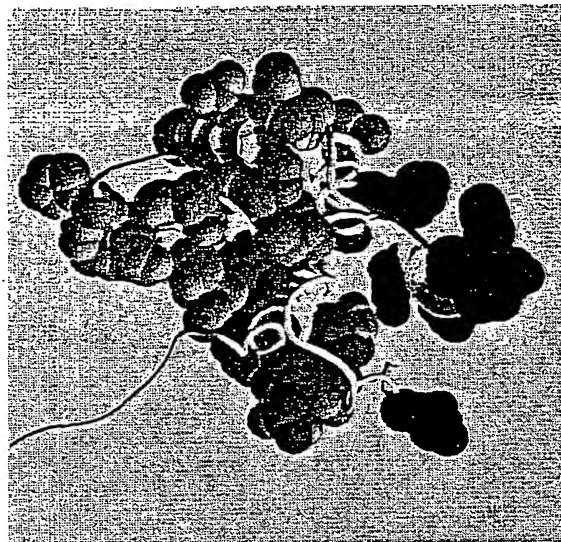


FIG.7A

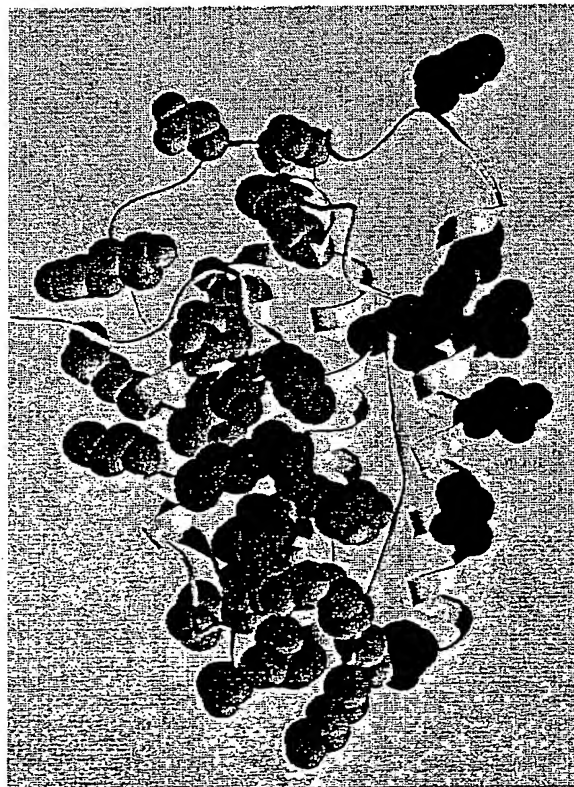


FIG.7B

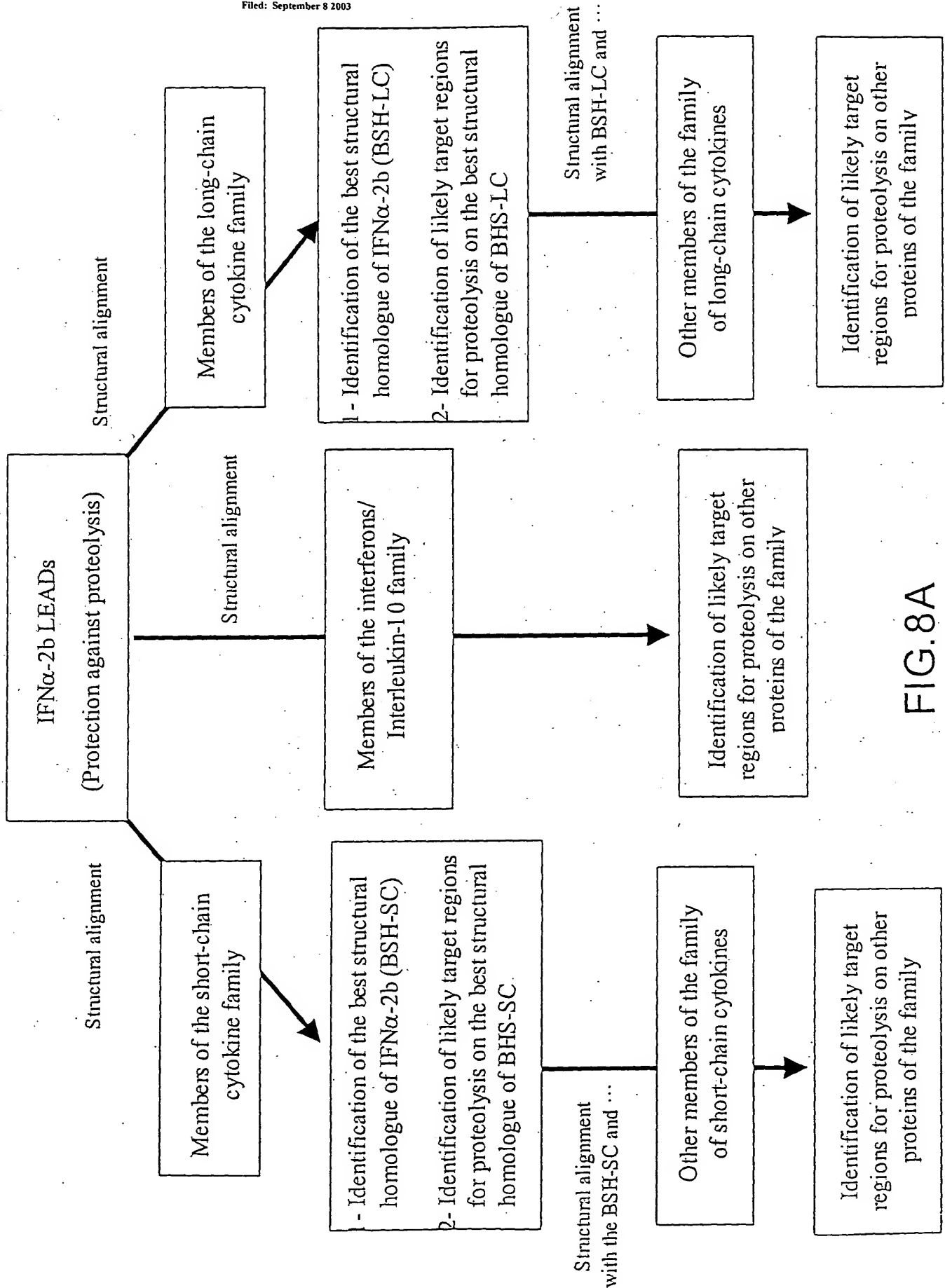


FIG.8A

Structural super-imposition of interferon  $\alpha$ -2b (1RH2)  
and interferon  $\beta$  (1AU1)

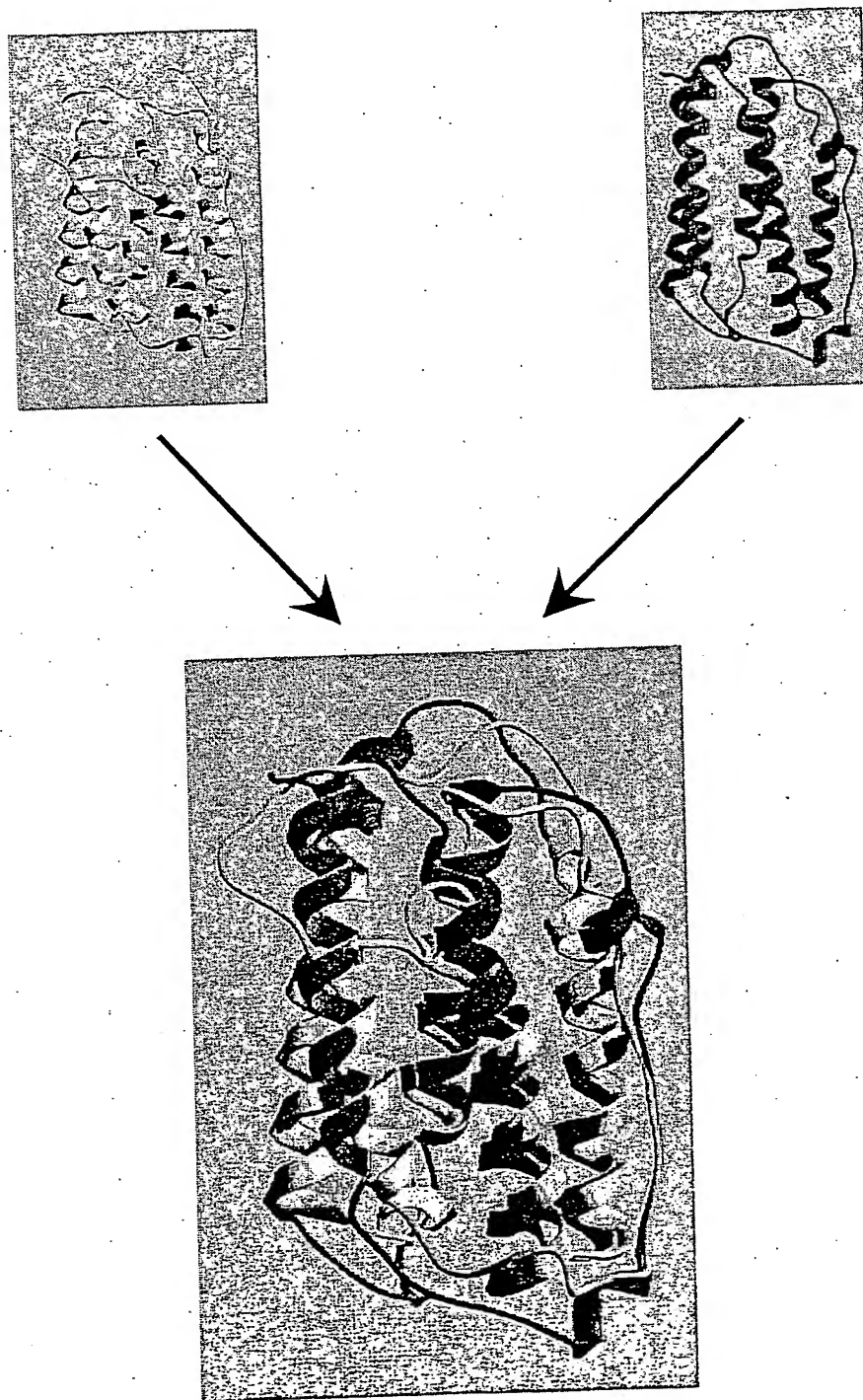


FIG.8B

Structural super-imposition of interferon  $\alpha$ -2b (1RH2)  
and erythropoietin (1BUY)

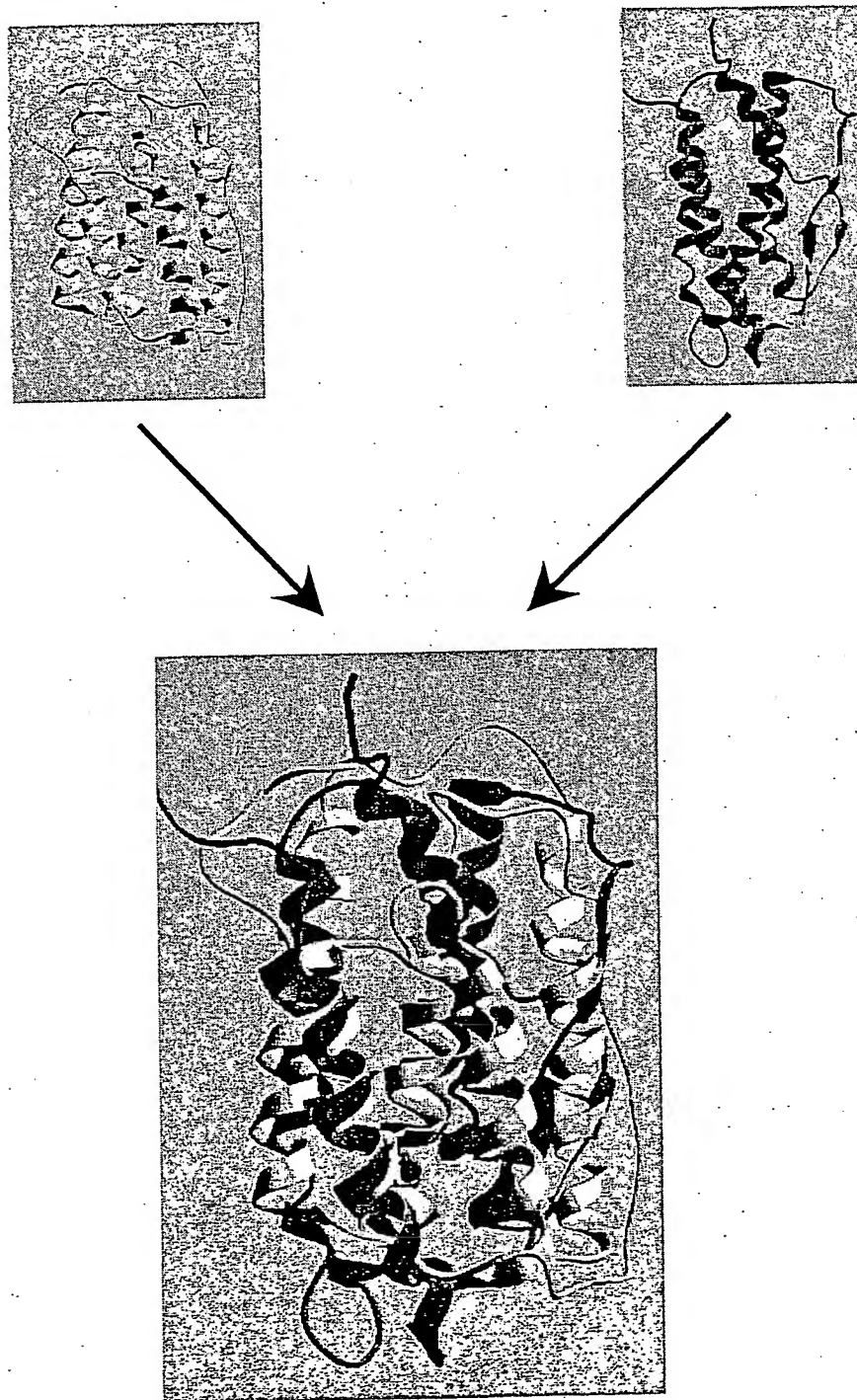


FIG.8C

Structural super-imposition of interferon  $\alpha$ -2b (1RH2)  
and granulocyte-colony stimulating factor (1CD9)

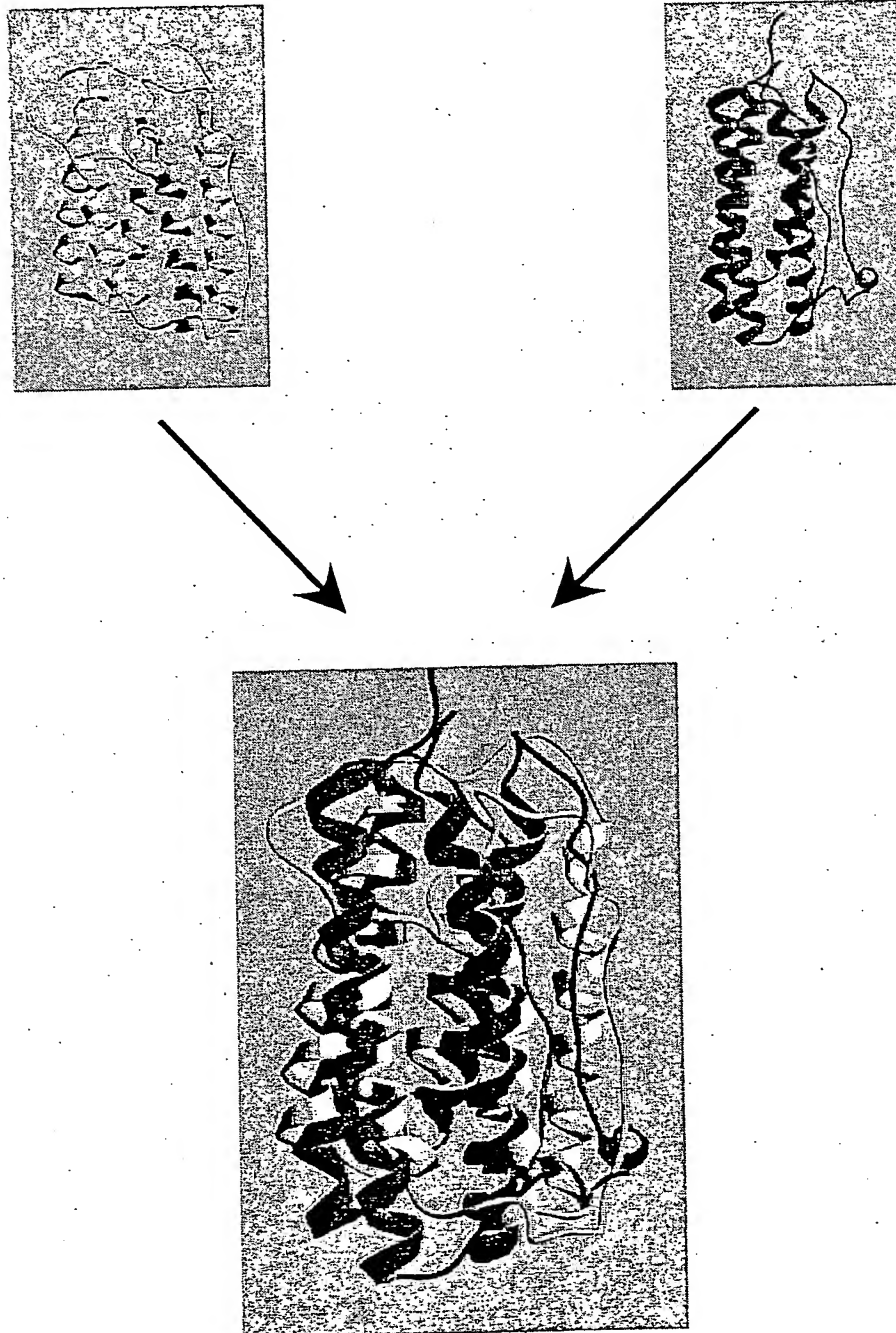


FIG.8D



Cytokine regions susceptible to protease attack identified by structural alignment with Lead mutants

of IFN  $\alpha$ -2b

IFN- $\alpha$ 2b	CDLPQTHSILGSRRTLMLLAQMRKI SLFSCILKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDLEACVIQG VGVTETPLMKEDSILAVRKYFQRIITLYLKEKKYSPCAWEVVRAEI MRSFSLSTNLQESLRSKE
Exemplary protein of the interferons/interleukin-10 family	
IFN- $\beta$	MSYNLIGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKOLOQFQKEDAAITIYEMLQNI FAI FRQDSSSTGWNETI VENLLANVYHQI NHLKTVLEEK LEKEDFTTRGKLMSSLHLKRYGRIILHYLKAKEYSHCAWTI VRVEILRNIFYFI NRI TGXLRN
Exemplary protein of the short-chain cytokines family	
EPO	APRRLICDSRVLERLYLLEAKEAENI TTGCAEHCSL NENITVPDTKVN FYAWKRMEVGQQA VEVMQGLALLSEAVLRGOALLVNSSQPWEPLQLHVDKAVSGLRSL TTLLRALGAQKEAISNSDAASAAPLRITI TADTFRKLFRVYSNPLRGKLKLYTGEACRTGDR
Exemplary protein of the long-chain cytokines family	
G-CSF	GPASSLPQSFLKLCLEQVRKI QGDGAALQEKLCATYKLCHPHEELVLGHSLGI PWAPLSSCP SQALQAGCLSQLHSGFLYQGLLQALEGISPELGPTLDTLQ DVADFATTI WQOMEELGMAPALQPTQGAMP AFASAFORRAGGVLVASHLQSFLEVS YRVLRLHAQP

FIG. 9

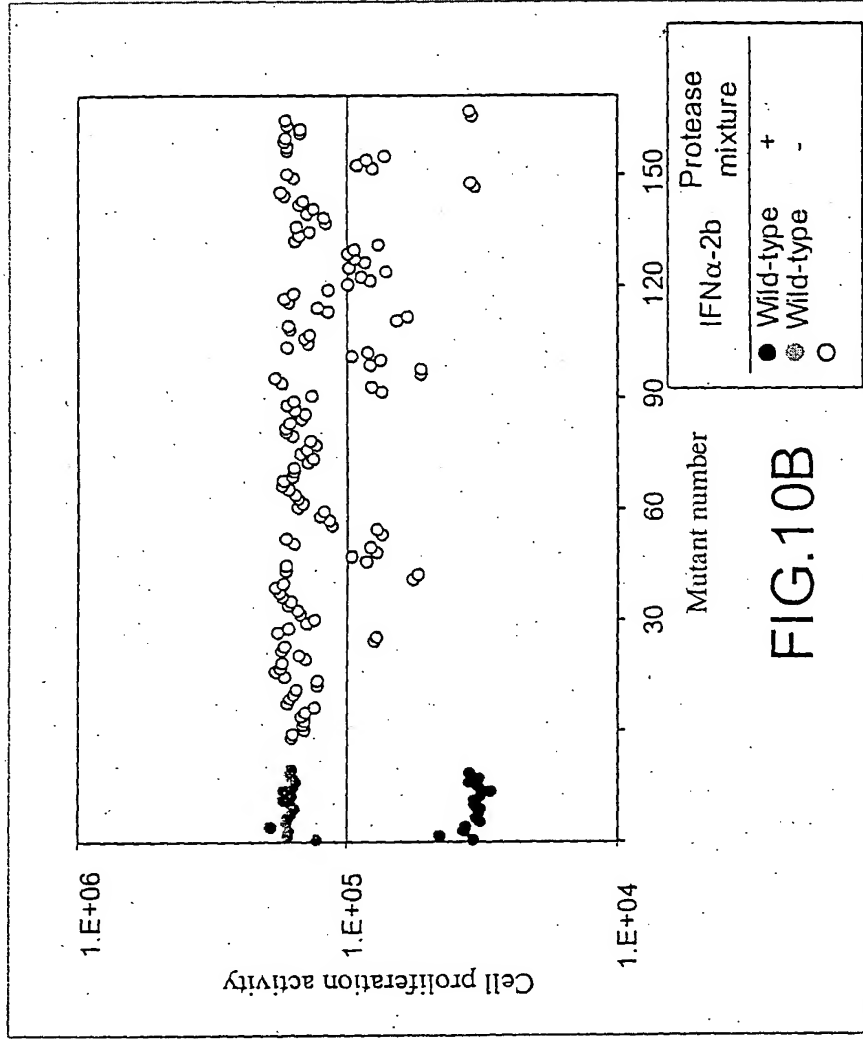
Antiviral activity (% of wild-type)

Mutant number

IFNα-2b	Protease mixture
●	+
◐	-
○	-

FIG.10A

Cell proliferation assay for alanine scanning of interferon  $\alpha$ -2b

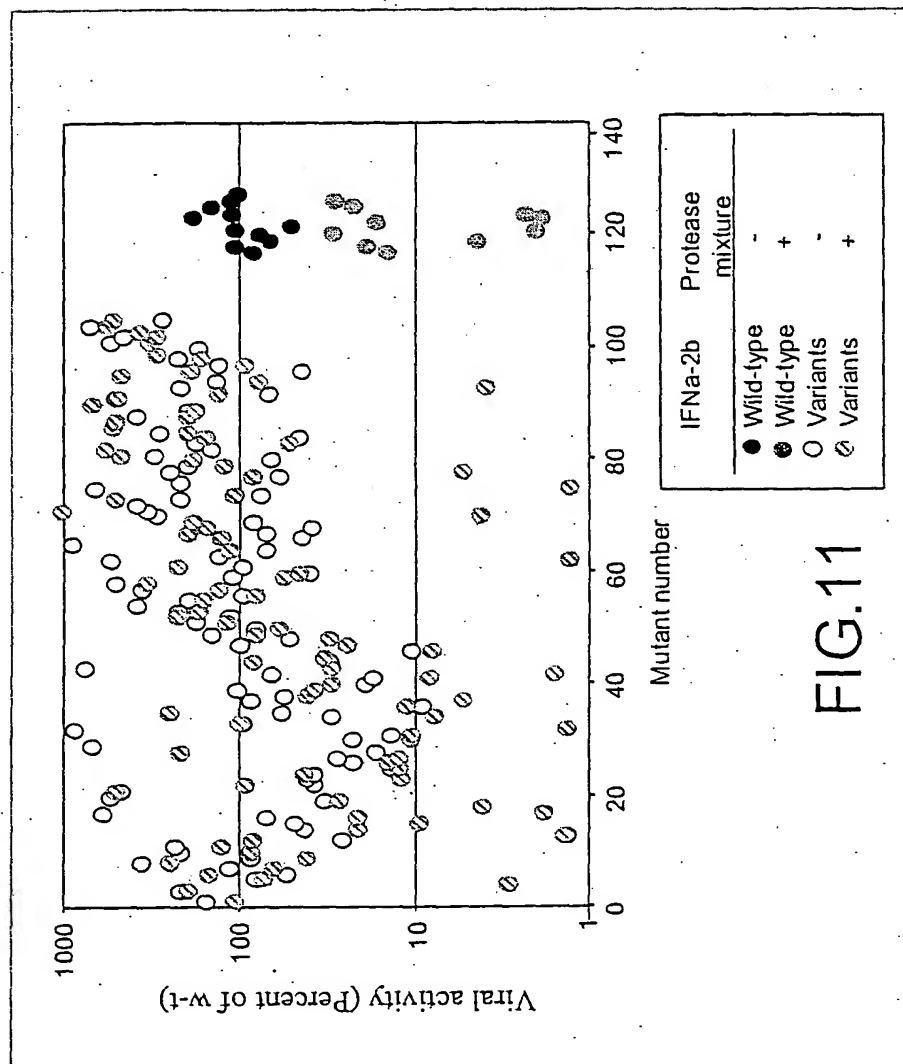


Scatter plot showing the relationship between Cell proliferation (X-axis) and Antiviral activity (% of wild-type) (Y-axis). The data points are represented by open circles, and a linear regression line is fitted to the data. The correlation coefficient is  $R = 0.9803$ .

The X-axis (Cell proliferation) ranges from  $2.0E+05$  to  $0.0E+00$ . The Y-axis (Antiviral activity (% of wild-type)) ranges from  $1.0E+0$  to  $1.0E+1$ .

FIG. 10C

Glycosylation of interferon  $\alpha$ -2b



## Interferon-beta

Protection against proteolysis

Sequence:

MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTI  
 YEMLQNI FAIFRQDSSSTGWN ETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMS  
 SLHLKRY YGRILHYLKAKEYSHCAWTIVRVEILRN FYFINRLTGYLRN

Exposed residues:

-----D--E--KQLQQ-QK-----  
 ----Q--FA--RQD-SS-G-NET-----EKEDF-R--L--  
 SLH-KR--GR-LH--KAKE-----Y-RN

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. D39Q	16. D73Q	31. F111I	46. L130I
2. D39N	17. D73N	32. F111V	47. K134Q
3. E42Q	18. E81Q	33. R113H	48. K134N
4. E42N	19. E81N	34. R113Q	49. K136Q
5. E42H	20. E81H	35. L116V	50. K136N
6. K45Q	21. E107Q	36. L116I	51. E137Q
7. K45N	22. E107N	37. L120V	52. E137N
8. L47V	23. E107H	38. L120I	53. E137H
9. L47I	24. K108Q	39. K123Q	54. Y163H
10. K52Q	25. K108N	40. K123N	55. Y163I
11. K52N	26. E109Q	41. R124H	56. R165H
12. F67I	27. E109N	42. R124Q	57. R165Q
13. F67V	28. E109H	43. R128H	
14. R71H	29. D110Q	44. R128Q	
15. R71Q	30. D110N	45. L130V	

FIG. 12A

# Interferon-gamma

Protection against proteolysis

Sequence:

CYCQDPYVKEAENLKKEYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKL

FKNFKDDQSIQKSVETIKEDMNVKFFNSNKKKRDDFEKLTN

Exposed residues:

-----T--L---KN-KEE-----K-  
 -KN-KDDQS-----

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

- |          |          |
|----------|----------|
| 1. L33V  | 12. E42H |
| 2. L33I  | 13. K58Q |
| 3. K37Q  | 14. K58N |
| 4. K37N  | 15. K61Q |
| 5. K40Q  | 16. K61N |
| 6. K40N  | 17. K64Q |
| 7. E41Q  | 18. K64N |
| 8. E41N  | 19. D65Q |
| 9. E41H  | 20. D65N |
| 10. E42Q | 21. D66Q |
| 11. E42N |          |

FIG. 12B

## Interleukin-10

Protection against proteolysis

Sequence:

SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFQMKDQLDNLLLKESLLEDFKGY  
 LGCQALSEMIQFYLEEVMPPQAENQDPDIKAHVNSLGENLKT

Exposed residues:

-----KESLLEDFKGY  
 L-----EM-QFY-EEV-PQ-ENQDPD-----K-

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. K49Q	18. K57N	35. E75Q
2. K49N	19. Y59H	36. E75N
3. E50Q	20. Y59I	37. E75H
4. E50N	21. L60V	38. P78S
5. E50H	22. L60I	39. P78A
6. L52V	23. E67Q	40. E81Q
7. L52I	24. E67N	41. E81N
8. L53V	25. E67H	42. E81H
9. L53I	26. M68V	43. D84Q
10. E54Q	27. M68I	44. D84N
11. E54N	28. F71I	45. P85S
12. E54H	29. F71V	46. P85A
13. D55Q	30. Y72H	47. D86Q
14. D55N	31. Y72I	48. D86N
15. F56I	32. E74Q	49. K88Q
16. F56V	33. E74N	50. K88N
17. K57Q	34. E74H	

FIG. 12C



# Ciliary neurotrophic factor

Protection against proteolysis

Sequence:

DSADGMPVASTDQWSELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAI  
 HTLLLQVAAFAYQIEELMILLEYKIPRNEADGMPINVGDGGLFEKKLWGLKVLQELSQQ  
 TVRSIHDLRFISSHOTGIPA

Exposed residues:

-----VASTDQWSELT-----Q---T-HVL-AR--E--QVH--PTEGD-----  
 -----EYKIPRNE-DGMPINVGDG-L-----  
 -----

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. D62Q	16. E92H	31. P135S
2. D62N	17. P100S	32. P135A
3. W64S	18. P100A	33. R136H
4. W64H	19. E102Q	34. R136Q
5. E66Q	20. E102N	35. E138Q
6. E66N	21. E102H	36. E138N
7. E66H	22. D104Q	37. E138H
8. L67V	23. D104N	38. D140Q
9. L67I	24. E131Q	39. D140N
10. L86V	25. E131N	40. P143S
11. L86I	26. E131H	41. P143A
12. R89H	27. Y132H	42. D148Q
13. R89Q	28. Y132I	43. D148N
14. E92Q	29. K133Q	44. L151V
15. E92N	30. K133N	45. L151I

FIG. 12D

# Granulocyte-colony stimulating factor

Protection against proteolysis

Sequence:

VLLGHSLGIPWAPLSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTL  
 QLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVS  
 RVLRLHLAQP

Exposed residues:

-----W-P-SS-PSQALQ-----S--F-----Q--E--PE-----  
 -----G-APALQPTQ-AM-A-ASAF-----  
 R--RH--QP-

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. W61S	12. E96N	23. P135S
2. W61H	13. E96H	24. P135A
3. P63S	14. P100S	25. F147I
4. P63A	15. P100A	26. F147V
5. P68S	16. E101Q	27. R169H
6. P68A	17. E101N	28. R169Q
7. L72V	18. E101H	29. R172H
8. L72I	19. P131S	30. R172Q
9. F86I	20. P131A	31. P177S
10. F86V	21. L133V	32. P177A
11. E96Q	22. L133I	

# Human growth hormone

Protection against proteolysis

Sequence:

SLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSN  
 VYDLLKDLEEGIQTLMGRLLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRK  
 DMDKVETFLRIVQCRSVEGSCGF

Expose residues:

-----ES-PT-SNREE-----E--QF-RS--AN-L-----  
 -----EDG-PRT-Q--KQTY-KFD-----  
 -----RS-EGSCG-

Proteases: ['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline  
 endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. E56Q	17. F92I	33. K140N
2. E56N	18. F92V	34. Y143H
3. E56H	19. R94H	35. Y143I
4. P59S	20. R94Q	36. K145Q
5. P59A	21. L101V	37. K145N
6. R64H	22. L101I	38. F146I
7. R64Q	23. E129Q	39. F146V
8. E65Q	24. E129N	40. D147Q
9. E65N	25. E129H	41. D147N
10. E65H	26. D130Q	42. R183H
11. E66Q	27. D130N	43. R183Q
12. E66N	28. P133S	44. E186Q
13. E66H	29. P133A	45. E186N
14. E88Q	30. R134H	46. E186H
15. E88N	31. R134Q	
16. E88H	32. K140Q	

FIG. 12F

## Interleukin-12

Protection against proteolysis

Sequence:

DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMALCLSSIYEDL  
 KMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYK  
 TKIKLCILLHAFRIRAVTIDRVMSYLNAS

Exposed residues:

-----KT--VE----LELTKNES-LNSRETSF-TNGSCLA-RK-----E--  
 KM--VE-KT-N---LM-PKR-----  
 -----R--S--NAS--

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. K56Q	15. E72Q	29. R92H	43. K117Q
2. K56N	16. E72N	30. R92Q	44. K117N
3. E61Q	17. E72H	31. K93Q	45. L124V
4. E61N	18. L75V	32. K93N	46. L124I
5. E61H	19. L75I	33. E107Q	47. M125V
6. L66V	20. R78H	34. E107N	48. M125I
7. L66I	21. R78Q	35. E107H	49. P127S
8. E67Q	22. E79Q	36. K110Q	50. P127A
9. E67N	23. E79N	37. K110N	51. K128Q
10. E67H	24. E79H	38. M111V	52. K128N
11. L68V	25. F82I	39. M111I	53. R129H
12. L68I	26. F82V	40. E115Q	54. R129Q
13. K70Q	27. L89V	41. E115N	55. R189H
14. K70N	28. L89I	42. E115H	56. R189Q

FIG. 12G

## Interleukin-6

Protection against proteolysis

Sequence:

SSKEALAENNLNLPKMAEKDGCFSQSGFNEETCLVKIITGLLEFEVYLEYLQNRFSSEE  
 QARAVQMSTKVLIIQFLQKKAKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTHLILRS  
 FKEFLQSSLRALRQM

Exposed residues:

-----PKMAEK-----FQSGF-----T---E-----E---QNR-ES-E-  
 -----DA-TTPDPTT-AS--TK-QAQNQW-----  
 -----R--RQM

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',

'Staphylococcal P.']

Exclusion list:['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. P64S	16. E92Q	31. D133N
2. P64A	17. E92N	32. P138S
3. K65Q	18. E92H	33. P138A
4. K65N	19. E98Q	34. D139Q
5. M66V	20. E98N	35. D139N
6. M66I	21. E98H	36. P140S
7. E68Q	22. R103H	37. P140A
8. E68N	23. R103Q	38. K149Q
9. E68H	24. E105Q	39. K149N
10. K69Q	25. E105N	40. W156S
11. K69N	26. E105H	41. W156H
12. F73I	27. E108Q	42. R178H
13. F73V	28. E108N	43. R178Q
14. F77I	29. E108H	44. R181H
15. F77V	30. D133Q	45. R181Q

FIG. 12H

# Leptin

Protection against proteolysis

Sequence:

VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGLDFIPGLHPILTLKMDQTLA  
 VYQQILTSMPSRNVIQISNDLENLRDLLHVLAFFSKSCHLPWASGLETLDLGGVLEASG  
 YSTEVVALSRLQGSLLQDMLWQLDLSPGC

Exposed residues:

-----P--H-IL-----  
 -----SCH-PW-SGLETLDS--GV-----  
 -----DLS-GC

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

- |           |           |
|-----------|-----------|
| 1. P43S   | 12. E105N |
| 2. P43A   | 13. E105H |
| 3. L49V   | 14. L107V |
| 4. L49I   | 15. L107I |
| 5. P99S   | 16. D108Q |
| 6. P99A   | 17. D108N |
| 7. W100S  | 18. D141Q |
| 8. W100H  | 19. D141N |
| 9. L104V  | 20. L142V |
| 10. L104I | 21. L142I |
| 11. E105Q |           |

# Leukemia inhibitory factor

Protection against proteolysis

Sequence:

PFPNNLDKLCGPNVTDFPPFHANGTEKAKLVELYRIVVYLGTS LGNITRDQKILNPSAL  
 SLHSKLNATADILRGLLSNVLCRLCSKYHVGHVDTVYGPDTSGKDV FQKKKLGCQLLGK  
 YKQIIAVLAQAF

Exposed residues:

-----PFHAN-T-----R-----T-----R--KIL-PS-  
 LS-----YH-GHVDVTYGPD-SGKDV F-----  
 -----Q---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. P69S	12. L104I	23. P148S
2. P69A	13. P106S	24. P148A
3. F70I	14. P106A	25. D149Q
4. F70V	15. L109V	26. D149N
5. R85H	16. L109I	27. K153Q
6. R85Q	17. Y137H	28. K153N
7. R99H	18. Y137I	29. D154Q
8. R99Q	19. D143Q	30. D154N
9. K102Q	20. D143N	31. F156I
10. K102N	21. Y146H	32. F156V
11. L104V	22. Y146I	

# Oncostatin M

Protection against proteolysis

Sequence:

ERPGAFPSEETLRGLGRRGFLQTLNATLGCVLHRLADLEQRLPKAQDLERSGLNIEDLE  
 KLQMARPNILGLRNNIYCMAQLLDNSDTAEPTKAGRGASQP

Exposed residues:

-----SEET-RGLG-----NA---C--HR-AD-EQR--KAQD-ERSGLNIE---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. E59Q	12. R84Q	23. D97N
2. E59N	13. D87Q	24. E99Q
3. E59H	14. D87N	25. E99N
4. E60Q	15. E89Q	26. E99H
5. E60N	16. E89N	27. R100H
6. E60H	17. E89H	28. R100Q
7. R63H	18. R91H	29. L103V
8. R63Q	19. R91Q	30. L103I
9. L65V	20. K94Q	31. E106Q
10. L65I	21. K94N	32. E106N
11. R84H	22. D97Q	33. E106H



## Erythropoietin

Protection against proteolysis

Sequence:

APRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNIFYAWKRMEVGQQ  
 AVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTLLRALGAQKEA  
 ISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKCLKLYTGEACRTGDR

Exposed residues:

-----N-T--DTKVNIFYA-KR-EV---  
 -----A--SE--LR-QA--VNSSQ-----  
 ISPPDA-SAAPLR-IT-----RTGDR

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. D43Q	14. E55N	27. L130V
2. D43N	15. E55H	28. L130I
3. K45Q	16. E72Q	29. R131H
4. K45N	17. E72N	30. R131Q
5. F48I	18. E72H	31. R162H
6. F48V	19. L75V	32. R162Q
7. Y49H	20. L75I	33. D165Q
8. Y49I	21. R76H	34. D165N
9. K52Q	22. R76Q	35. P121S
10. K52N	23. D123Q	36. P121A
11. R53H	24. D123N	37. P122S
12. R53Q	25. P129S	38. P122A
13. E55Q	26. P129A	

# Flt3 ligand

Protection against proteolysis

Sequence:

TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWMER

LKTVAGSKMQGLLERVNTIEIHFVTKCAFQPPPSCLRFRVQTN

Exposed residues:

TQD-----T--S--QD-EL-----R--ER

-KTV-G-----A-QPPPSCLRFV---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',

'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	D3Q	15.	R59H
2.	D3N	16.	R59Q
3.	D40Q	17.	K61Q
4.	D40N	18.	K61N
5.	E42Q	19.	P89S
6.	E42N	20.	P89A
7.	E42H	21.	P90S
8.	L43V	22.	P90A
9.	L43I	23.	P91S
10.	R55H	24.	P91A
11.	R55Q	25.	R95H
12.	E58Q	26.	R95Q
13.	E58N	27.	F96I
14.	E58H	28.	F96V

FIG. 12M

## Granulocyte-macrophage colony-stimulating factor

Protection against proteolysis

Sequence:

APARSPSPSTQPWEHVNAIQEARRLLNLSRDTAAEMNETVEVISEMFDLQEPTCLQTRL  
 ELYKQGLRGS�TKLKGPLTMMASHYKQHCPTPETSCATQIITFESFKENLKDFLLVIP  
 FDCWEPVQE

Exposed residues:

-----ET-E--SEM-DLQE-----  
 E--KQ--R-----PETSCATQI-T-----  
 FD--EP---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. E38Q	14. L49V	27. P92A
2. E38N	15. L49I	28. E93Q
3. E38H	16. E51Q	29. E93N
4. E41Q	17. E51N	30. E93H
5. E41N	18. E51H	31. F119I
6. E41H	19. E60Q	32. F119V
7. E45Q	20. E60N	33. D120Q
8. E45N	21. E60H	34. D120N
9. E45H	22. K63Q	35. E123Q
10. M46V	23. K63N	36. E123N
11. M46I	24. R67H	37. E123H
12. D48Q	25. R67Q	38. P124S
13. D48N	26. P92S	39. P124A

FIG. 12N

# Interleukin-13

Protection against proteolysis

Sequence:

GPVPPSTALRELIEELVNITQNQKAPLCNGSMVWSINLTAGMYCAALESLINVSGCSAI

EKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLFREGRFN

Exposed residues:

-----M-WS-NLTAG-----E--INVSG-----

-----AGQFSSLHVRDTK-----REGRFN

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',

'Staphylococcal P.']

Exclusion list:['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. M32V	11. F79V	21. R107Q
2. M32I	12. L82V	22. E108Q
3. W34S	13. L82I	23. E108N
4. W34H	14. R85H	24. E108H
5. L38V	15. R85Q	25. R110H
6. L38I	16. D86Q	26. R110Q
7. E48Q	17. D86N	27. F111I
8. E48N	18. K88Q	28. F111V
9. E48H	19. K88N	
10. F79I	20. R107H	

## Interleukin-2

Protection against proteolysis

Sequence:

APTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL  
 EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL  
 NRWITFCQSIISTLT

Exposed residues:

-----K-Y--KKATEL---Q--  
 EE--KP-EE--NL-----ETTFM-EYADET-T-----  
 -----STLT

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. K43Q	13. L53I	25. E68Q	37. E106Q
2. K43N	14. E60Q	26. E68N	38. E106N
3. Y45H	15. E60N	27. E68H	39. E106H
4. Y45I	16. E60H	28. L72V	40. Y107H
5. K48Q	17. E61Q	29. L72I	41. Y107I
6. K48N	18. E61N	30. E100Q	42. D109Q
7. K49Q	19. E61H	31. E100N	43. D109N
8. K49N	20. P65S	32. E100H	44. E110Q
9. E52Q	21. P65A	33. F103I	45. E110N
10. E52N	22. E67Q	34. F103V	46. E110H
11. E52H	23. E67N	35. M104V	47. L132V
12. L53V	24. E67H	36. M104I	48. L132I

FIG. 12P

## Interleukin-3

Protection against proteolysis

Sequence:

APMTQTTPLKTSWVNCSNMIDEIITHLKQPPLPLLDNFNNLNGEDQDILMENNLRPNLE  
 AFNRAVKSLQNASAIESILKNLLPCLPLATAAPTRHPIHIKGDWNEFRRKLTFYLKTL  
 ENAQAAQQTTLSLAIF

Exposed residues:

-----F-N-NGE-QD-----E  
 ---R---KS-Q-----HP-H-KD-D-----  
 -----

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

- |          |           |
|----------|-----------|
| 1. F37I  | 12. R63Q  |
| 2. F37V  | 13. K66Q  |
| 3. E43Q  | 14. K66N  |
| 4. E43N  | 15. P96S  |
| 5. E43H  | 16. P96A  |
| 6. D46Q  | 17. K100Q |
| 7. D46N  | 18. K100N |
| 8. E59Q  | 19. D101Q |
| 9. E59N  | 20. D101N |
| 10. E59H | 21. D103Q |
| 11. R63H | 22. D103N |

FIG. 12Q

### Interleukin-4

Protection against proteolysis

Sequence:

HKCDITLQEI I KTLNSLTEQKTLCTELTVTDIFAASKNTEKETFCRAATVLRQFYSHH  
EKDTRCLGATAQQFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERLKT  
IMREKYSKCSS

Exposed residues:

-----E-T-----AASKNTT-----RQ--SH-  
EK-TR-L-----SCPVKEANQ-----  
-----KCSS

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

- |          |           |
|----------|-----------|
| 1. E26Q  | 14. R64Q  |
| 2. E26N  | 15. L66V  |
| 3. E26H  | 16. L66I  |
| 4. K37Q  | 17. P100S |
| 5. K37N  | 18. P100A |
| 6. R53H  | 19. K102Q |
| 7. R53Q  | 20. K102N |
| 8. E60Q  | 21. E103Q |
| 9. E60N  | 22. E103N |
| 10. E60H | 23. E103H |
| 11. K61Q | 24. K126Q |
| 12. K61N | 25. K126N |
| 13. R64H |           |

FIG. 12R

# Interleukin-5

Protection against proteolysis

Sequence:

IPTEIPTSALVKETLALLSTHRTLLIANETLRIPVPVHKNHQLCTEEIFQGIGTLESQT  
 VQGGTVERLFFKNLSLIKKYIDGQKKKCGEERRRVNQFLDYLQEF LGVMNTEWIIIES

Exposed residues:

-----R-P--V-K-----EE--Q--GT-ESQ-  
 -----KK-GEER-----E-----NTEW----

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. R32H	13. E56Q	25. E89H
2. R32Q	14. E56N	26. R90H
3. P34S	15. E56H	27. R90Q
4. P34A	16. K84Q	28. E102Q
5. K39Q	17. K84N	29. E102N
6. K39N	18. K85Q	30. E102H
7. E46Q	19. K85N	31. E110Q
8. E46N	20. E88Q	32. E110N
9. E46H	21. E88N	33. E110H
10. E47Q	22. E88H	34. W111S
11. E47N	23. E89Q	35. W111H
12. E47H	24. E89N	

FIG. 12S



# Stem cell factor

Protection against proteolysis

Sequence:

EGICRNRVTNNVKDVTKLVANLPKDYMITLKYVPGMDVLP SHCWISEMVVQLSDSLTDL  
 LDKFSNISEGLSNYSIIDKLVNIVDDLVECVKENSSKDLKKSFKSPEPRLFTPEEFFRI  
 FNRSIDAFKDFVVASETSDCVVS

Exposed residues:

-----M-T-K--P--DV-----V--D--TD--  
 -DKFSN-----SK-LKKSFKS-EPRL-----  
 -----ASETSDCVVS

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',  
 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '\*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. M27V	16. K62N	31. E106Q
2. M27I	17. F63I	32. E106N
3. K31Q	18. F63V	33. E106H
4. K31N	19. K96Q	34. P107S
5. P34S	20. K96N	35. P107A
6. P34A	21. L98V	36. R108H
7. D37Q	22. L98I	37. R108Q
8. D37N	23. K99Q	38. L109V
9. D54Q	24. K99N	39. L109I
10. D54N	25. K100Q	40. E134Q
11. D58Q	26. K100N	41. E134N
12. D58N	27. F102I	42. E134H
13. D61Q	28. F102V	43. D137Q
14. D61N	29. K103Q	44. D137N
15. K62Q	30. K103N	

FIG. 12T